Birla Central Library
PILANI (Jaipur State)

Class No :- 616.9
Book No :- B966V
Accession No :- 35261

HARVARD UNIVERSITY MONOGRAPHS IN MEDICINE AND PUBLIC HEALTH

Editorial Committee

John F. Enders
A. Baird Hastings
James Howard Means
S. Burt Wolbach

Executive Secretary

Katherine R. Drinker

LONDON : GEOFFREY CUMBERLEGE
OXFORD UNIVERSITY PRESS

VIRUS AS ORGANISM

Evolutionary and Ecological Aspects of Some Human Virus Diseases

BY

FRANK MacFARLANE BURNET, M.D., F.R.S.
Director, Walter and Eliza Hall Institute of Research
in Pathology and Medicine, Melbourne, Australia

HARVARD UNIVERSITY PRESS
Cambridge, Massachusetts
1946

COPYRIGHT, 1945
BY THE PRESIDENT AND FELLOWS OF HARVARD COLLEGE

Second Printing

PRINTED IN THE UNITED STATES OF AMERICA

HARVARD UNIVERSITY

THE EDWARD K. DUNHAM LECTURES

for the

PROMOTION OF THE MEDICAL SCIENCES

1944

PREFACE

IN GIVING the Dunham Lectures and in preparing this expansion of them, my object was to discuss some of the virus diseases of man from a consistently biological angle. Despite the great recent advances in knowledge of the physiochemical nature of viruses, there is no escape from the pragmatic necessity of regarding them as living organisms, reproducing their kind and subject to the same imperative of continued survival in a changing environment as other organisms. The development of such a point of view seems to be needed if the great mass of experimental and clinical work on virus disease is to be brought into a satisfactory relationship with general biological thought.

This attempt is necessarily very incomplete, and I have taken the privilege of a lecturer to discuss general questions very largely in terms of work with which I have been personally concerned and to indulge in speculation about origins and change in virus disease beyond what would be admissible in general scientific writing.

F. M. BURNET

Melbourne
October, 1944

CONTENTS

I. REPRODUCTION, VARIATION, AND SURVIVAL 3

II. EVOLUTION AND CHANGE IN VIRUS DISEASE 19

III. THE REACTION OF THE HOST TO VIRUS INFECTION . . . 36

IV. HERPES SIMPLEX 47

V. POLIOMYELITIS 57

VI. PSITTACOSIS AND RELATED INFECTIONS 69

VII. SMALLPOX, ALASTRIM, AND VACCINIA 80

VIII. YELLOW FEVER 94

IX. INFLUENZA 102

X. CONCLUSION 124

BIBLIOGRAPHY 128

VIRUS AS ORGANISM

I

REPRODUCTION, VARIATION, AND SURVIVAL

SCIENCE has two distinct modes of approach to the study of living organisms. On the one hand, we have those disciplines which deal essentially with the organism in isolation and which are chiefly concerned with its structure, chemical and morphological, and with its functioning as an individual unit. On the other hand, we have the sciences whose object is to interpret and control the phenomena of living things as they are, in such environments as the processes of nature or the activities of mankind have allotted them. This second group contains the ecological sciences concerned essentially with how organisms make a living and with the factors which determine how their numbers are distributed both in space and in time. It is only broadening the scope of ecology somewhat to make it cover as well the long-term historical aspects of the interaction between organism and environment, i.e., its evolution.

It may appear foolhardy to attempt any more than the most superficial discussion of viruses from such a point of view. It is true that we are only at the beginning of accurate knowledge either of their systematics or of their chemical nature and metabolic activities, but after all this is almost equally true of any other type of living organism. We know at least as much about tobacco mosaic virus or vaccinia virus as we do about the tobacco plant or the rabbit—considering the sizes of the organisms concerned, a great deal more. The very simplicity of viruses does in fact demand that a wholehearted attempt should be made to study the basic processes of reproduction, variation, and survival as they are manifested in the group. Such study will demand all the experimental data that can be obtained by physiochemical, clinical, and epidemiological techniques as well as by the methods of experimental pathology, but the picture must remain incomplete if it cannot be fitted into the pattern of evolutionary change that has been worked out for higher organisms. In this preliminary chapter an attempt will be made to discuss in what respects reproduction, mutation, and survival in viruses resemble and differ from these processes in higher organisms.

Reproduction of Viruses

The general character of the viruses, once the "filterable viruses," is now familiar to most biologists, but it may be advisable to give a brief outline of those of their characteristics which bear on the topics to be discussed in these lectures. In the earlier phases of their study, viruses could be defined as those agents of disease which could pass through a standard filter capable of holding back bacteria and which failed to multiply in any medium other than living cells of a susceptible host. This is still a good working definition, but the concentration of research on the viruses since about 1930 has made it difficult to provide a simple inclusive definition of the agents now included in the group. It is conventional to omit those organisms which can be conveniently studied by ordinary microscopic methods, the rickettsias and the smaller intracellular protozoa, though these may be as strictly limited to growth in the living cell as the viruses. On the other hand, the organisms of the psittacosis group in some phases are even larger than an average rickettsia but are included with the viruses.

All the viruses susceptible to detailed laboratory study have now been "measured" with more or less accuracy by the various types of physical technique which are applicable to such problems. Electron micrographs have in general satisfactorily confirmed the deductions made by more indirect physical methods. There are numerous reviews (1, 2) available as to the size and shape of virus particles, and here it is only necessary to state that they range in diameter from about 10 mμ to about 300 mμ, and that most of those responsible for animal infections are more or less spherical in form although there is some evidence that the virus of poliomyelitis is of slender rod shape.

The evidence as to chemical composition is limited to a few viruses only. Vaccinia virus appears to have almost as complex a chemical structure as a typical bacterium. Nucleoprotein, carbohydrate, and lipoid are present; the nucleic acid is of the desoxyribose type; and the lipoids include cholesterol, phospholipid, and neutral fat. Evidence of complex enzyme systems is given by the presence in the virus of biotin, flavine-adenine-dinucleotide, and copper (3). Less extensive work on influenza virus, rabbit papilloma virus, and the virus of equine encephalomyelitis indicates that they are predominantly nucleoprotein in composition. This has been more fully established for tobacco mosaic virus protein and two bacterial viruses (bacteriophages).

It is an interesting aspect of the history of biology that whenever there has been obscurity about the details of an organism's method of reproduction, two opposing hypotheses have always appeared. One group of biologists have held that the process was of the normal reproductive character, like producing like, while their opponents have displayed ingenuity in devising hypotheses of production *de novo* from unlike material living on non-living. Mice and swallows were generated in Nile mud and maggots from decaying meat. Simple experiments eventually disproved the wilder fallacies and by the middle of the nineteenth century the controversy was restricted to the question of the origin of bacteria in organic infusions. With the refutation of the hypothesis of spontaneous generation by Pasteur and Tyndall, bacteriology developed rapidly, and most of the mystery of bacterial origins and reproduction disappeared. When the existence of filterable viruses was recognised, the old controversy again arose, particularly in regard to the bacterial viruses (bacteriophages). Discussion centred around two alternatives: whether these disease-producing agents arose solely by genetic descent from similar agents or whether they were products of the host cells involved in the disease process. As knowledge of the viruses has accumulated it has become obvious (perhaps only within the last five years) that the question must be answered as all the preceding ones were answered. We can state dogmatically that there is no evidence whatever that any virus, whether its host be animal, plant, or bacterium, arises *de novo*. Every virus particle like any other organism derives by genetic descent from some similar particle, and in its turn possesses the power to produce under appropriate conditions replicas of itself.

In practice every experimental investigator and every public health administrator acts as though he were convinced that every virus particle is the direct descendant of pre-existing virus particles of the same general character. Rinderpest, the most deadly of all virus diseases of cattle, has only once entered Australia. Its recognition was followed by strict isolation of a wide area and the ruthless slaughter of all cattle within the area concerned. This drastic action was quite effective, but essentially it was an act of faith based on the belief that virus springs always from its like.

Virus diseases breed true to type in the same ways that protozoal or bacterial diseases do. The classical example of constancy of disease type through the centuries is a virus disease. Mumps was described by Hippocrates in terms which make it certain that clinically and epidemiologically the disease was identical with that we see today. For more than 2500 years, the virus has passed without significant change from one susceptible individual to another. Millions of such transfers went no further, but a sufficient number of continuing chains of infection remained to perpetuate the species.

Change in character of virus disease is equally evident in the epidemiological records. Smallpox, measles, influenza, and yellow fever have all shown as striking changes in virulence and distribution within the historical period as have diphtheria, scarlet fever, syphilis, and malaria. In fact, one of the major impressions one obtains from epidemiological history is the uniformity in diversity of the important epidemic diseases irrespective of the type of pathogen now known to be responsible. Where the reported picture of clinical and epidemiological happenings does not correspond closely to any modern condition, we can be wholly at a loss to say whether the agent was protozoal, bacterial, or virus.

The plague of Athens in 430–425 B.C. was described in great detail by Thucydides but has been variously ascribed to plague, scarlet fever, typhus, yellow fever, smallpox, and melioidosis.

The possibility that virus-like agents might, under certain circumstances, be liberated from the cells of higher animals or plants has not been wholly abandoned by some authors. In one sense the work of Griffith, Avery, and others on the transformation of pneumococcal types proves that a relatively simple chemically defined agent (a thymonucleic acid) can behave in a virus-like fashion, and one cannot be certain that future work on neoplastic diseases may not bring to light similar agents. But at present no such example has been demonstrated. In 1938 Doerr (4) considered it probable that herpes virus was a product of certain human cells stimulated to abnormal activity by the various bodily changes which are known to provoke the clinical manifestations of herpes simplex. In a subsequent chapter the contrary evidence for the view that herpes virus is as typical an extrinsic virus as that of chickenpox or poliomyelitis will be given. As far as a study of recent literature from countries outside of continental Europe and Japan allows one to state, there are at present no supporters of Doerr's contention.

The nature of bacterial viruses (bacteriophages) was for twenty years a matter of controversy with the greater number of authors, probably influenced by the authority of Bordet, in favour of the view that they were not autonomous viruses. Northrop and Krueger and later Krueger and his collaborators upheld the view that phages were liberated from bacteria by the activation of a precursor normally present within the bacterial cell. There has been little fundamental study of bacteriophage phenomena in the last few years, and it is perhaps premature to say that unanimity has been reached in favour of regarding bacteriophages simply as bacterial viruses. Nevertheless, it is fair to say that the contributions of d'Herelle, Burnet, Elford and Andrewes, Schlesinger, and Luria and Delbrück in favour of this view have not been refuted, and that recent electron micrographs showing the characteristic morphology of the larger particles have convinced most of those interested in the subject that bacteriophages are essentially similar to other viruses.

There has been also an interesting change of accent in recent years in regard to the controversy as to whether the smaller viruses are to be regarded as protein molecules or living microorganisms. The dilemma has been generally recognised as an unreal one, and it seems more than likely that future work will show that protein production, a strictly biological activity, will eventually be understood in terms of replica production by subcellular, essentially living entities (5). The production of virus protein in the infected cell will then represent only one special example of protein synthesis in general. Such a point of view is at present only directly applicable to some of the plant viruses, but, using very general terms, it is legitimate to regard all reproduction of viruses as essentially a process of replica production within the living susceptible host cell. The material atoms and the energy necessary for replica production are drawn in some way from the metabolic activities of the cell. The process is no more and no less easily understood than the replication of cell structures in mitosis.

It is probable that different viruses differ greatly in the complexity of the replica production process. Rickettsias and the larger viruses clearly have a more complex structure than the smaller ones and probably include intrinsic enzyme systems lacking in the latter. Their multiplication may well resemble in many respects that of the more delicate pathogenic bacteria. As yet there are no details available as to the nature of the interaction between virus and susceptible cell. As every virus worker knows, there are curious limitations in regard to the range of cells that can support multiplication of a given virus or show the characteristic pathological changes indicative of multiplication. There are, at the same time, increasing indications that the concepts of clinical and subclinical infection must be extended to the cellular as well as to the individual level. The phenomena of interference suggest strongly that under certain conditions cells can be invaded by virus particles without subsequent multiplication but with the appearance of resistance to invasion by more virulent virus particles. There are insistent hints that as a preliminary to multiplication some specific pseudo-immunological complementary relation between virus surface and some cell constituent is needed, and there are other indications that rapidly metabolizing (embryonic) cells support multiplication more readily than inactive ones of similar type. Beyond these hints we have no knowledge of the intracellular conditions necessary for multiplication or of the conditions which on occasion can inactivate or destroy virus within the cell.

VARIATION IN VIRUSES

Variability is so characteristic of viruses that it is hard to find a paper which fails to mention some aspect of virus variation. It has been standard practice since Pasteur developed a "fixed" strain of rabies virus for any worker with a human virus to "adapt" it to whatever experimental animal he has chosen. The first step in his research programme has normally been to change the character of the virus so that, instead of giving irregular symptoms or lesions in the foreign host used experimentally, the virus will give a reproducible fatal infection with an approximately constant titration endpoint. Such procedures must be based on knowledge or faith that a virus is a labile entity, the average character of whose component units can be changed by suitable manipulations. Most such work seems to have been done without much attempt to define what happens in the production of altered "fixed" strains. The general impression one receives is that "the virus" (a rather mystically conceived entity that in some way unifies and is more than its component generations of virus particles) is trained to multiply in and damage the cells of the new host by the process of continued animal-to-animal passage—an essentially Lamarckian conception.

Such ideas are not in key with contemporary biological thought. As far as one who is not a geneticist can gather from reviews and general discussions of evolutionary problems, notably from J. S. Huxley's writings (6), the current tendency is almost wholly toward an essentially Darwinian view. The raw material of evolution is provided primarily by gene-mutations but also to a considerable extent by grosser chromosomal changes—duplication, inversion, or loss of a segment of a single chromosome, doubling of the normal number of chromosomes, or increase in the number by some other mechanism. The manifestations of these changes in individuals and in the species as a whole are governed by many factors too complex to discuss here. Genetics is essentially, or by some definitions wholly, concerned with bisexual reproduction, and most of its concepts have been developed from experimental hybridization between similar forms. Only a very limited portion of the subject is, therefore, applicable to the asexual type of reproduction universal amongst the bacteria and viruses. We need mention only one or two of the newer points of view amongst geneticists as possibly relevant to problems arising from microorganismal variation. In the first place, it is no longer possible to relate a given gene to a given character; the gene is responsible not for the character but for a character-difference. Even with this limitation the effect of a given gene on the character in question may differ according to differences in the environment or according to differences in the remainder of the genetic mechanism. Another important development has been the recognition that the genetic mechanism itself is a product of evolution, and that in any particular group of organisms it has become adapted to make the "best" use (as always for survival) of such mutations as arise. In general, any mutation is rapidly "buffered" by associated changes in genes and gene combinations which can act as "modifiers" for the mutating gene. When a distinct pathological mutant of, e.g., *Drosophila*, is obtained, continued inbreeding will usually bring the character of the strain more or less back to the parent type, though suitable outcrossing will show the continued existence of the original gene mutation.

Every part of the genetic representation in the chromosomes, every gene, is potentially subject to various significant types of chemical rearrangement, induced either by the incidence of ionizing radiations or by processes which at present can only be described as accidental. With such intensively studied organisms as *Drosophila* or maize, gene mutations occur in predictable fashion, some more frequently than others. For any of the commoner easily detectable types of change, an approximate figure can be given of the rate of spontaneous appearance and of the rate when exposed to, e.g., X-rays of certain intensity. In several instances, the spontaneous mutation rate has been of the order 1 in 10^5; i.e., of 100,000 progeny of a given strain, one individual will show the mutant character. Other types of mutation, however, must occur with much less frequency. Wild drosophila populations show genetic differences of the same character as those observed in laboratory strains, and the mutation rate in the field is apparently of the same order. There is no reason to believe that the position with all other sexually reproducing organisms is in any essential sense different from that in *Drosophila.*

The study of variation in organisms reproducing asexually, such as bacteria and viruses, has not yet led to any compact body of generalization at all comparable to that of classical genetics. An immense amount of work has been done on bacterial variation ever since Pasteur's demonstration that attenuated variants might be used to immunize animals against a more virulent strain of the corresponding bacterial type. Most of such work has been done by methods which provide no data as to the frequency with which mutants appear nor any evidence as to whether the variant finally obtained was a primary mutant from the parent strain or appeared only as a result of a progressive series of heritable changes. There is a general tendency in the literature to avoid expressing an opinion even as to whether the variations observed result essentially from discontinuous mutation with selective survival of variants particularly suitable to the abnormal experimental environment, or whether they are to be regarded as modifications produced by a direct somatic effect (Lamarckian) of the changed environment.

There has, however, been a certain amount of work on heritable bacterial changes which are known to be primary mutations from the parent form. The first type of bacterial variant studied as a problem in itself was the lactose-fermenting variant of a certain coliform bacillus which, under the influence of de Vries's mutation theory of evolution was called by Massini (7) *B. coli mutabile*. When this organism is plated on one of the ordinary differential media for intestinal bacilli, colorless colonies appear. If the plate is incubated for some days, red (lactose-fermenting) papillae appear in some of the colonies, and from these papillae consistently lactose-fermenting organisms can be subcultured. More recent study of such a strain has shown that the change from non-lactose-fermenting to lactose-fermenting is a discontinuous change involving about one organism in 500,000. Lewis's (8) figures for five distinct substrains grown on lactose-free media ranged from 1:250,000 to 1:600,000. Detailed study of the enzymes responsible for lactose splitting indicates that in all probability the actual change is not the sudden appearance of a new enzyme but a change in the bacterial surface rendering it permeable to lactose. This, however, in no way discounts the evidence that the change is discontinuous and occurs with an approximately constant frequency of the order $1{:}10^5$–10^6.

The recognition of the "smooth" (S) and "rough" (R) forms of many bacterial species by De Kruif (9) and Arkwright (10) led to an extensive study of the phenomena involved. It was shown that the "rough" variants were essentially organisms which had lost genetically the power to produce the specific polysaccharide responsible for many of the properties of the normal pathogenic S form. R forms were normally obtained by allowing broth cultures to age, and no estimate could, therefore, be made as to the proportion of variants arising or the way in which they arose. In 1927 (11) I made some experiments with bacteriophages limited in their action to S or R forms respectively of certain salmonella strains. By spreading a (young diluted) S broth culture over a plate with an excess of specifically S phage, all the normal S organisms were inhibited. Only colonies of R forms or of phage-resistant S forms developed. The number of R colonies could be readily counted and proved to be proportional to the number of S organisms plated and independent of the concentration of bacteriophage used. The only interpretation of these results which seemed admissible was that the S–R change was a spontaneous discontinuous mutation occurring with a frequency of the order $1:10^6$.

As has been found in other types of experiment, recently isolated R strains can give rise to S strains; and the reverse experiment, using a specifically R phage on an R culture, gave S colonies with essentially the same frequency. Luria and Delbrück (12) have recently made a more elaborate study of the appearance of phage-resistant variants of a strain of coliform bacilli. They reached a similar conclusion that such variants arose by spontaneous mutation irrespective of the action of the phage. In their case the mutation rate per bacterial generation was of the order 2.5×10^{-8}.

It will be noticed that in both these types of bacterial variation there are available methods for detecting and enumerating the very small proportion of the population which show the mutant character under study. Only when such methods are available is it possible to determine, as in these cases, that the mutant form appears spontaneously and with an approximately constant frequency.

In the virus field similar methods could be applied to the study of variation in bacteriophages, but no systematic work seems to have been reported. An example of discontinuous mutation in a bacteriophage was described by Burnet and Lush (13), but the study was concerned mainly with qualitative characteristics, and only a rough hint of the order of frequency of the mutation can be drawn from their results.

There are great technical difficulties in applying these principles to the study of animal pathogenic viruses. In the discussion of influenza virus (p. 110), an example is given in which specially favourable circumstances made it possible to show that at least one type of discontinuous variation takes place in influenza virus A, and to make a rough estimate of the frequency with which the mutant type appeared. It is probable that there are a number of other instances of virus variation—e.g., when infectivity for two different hosts is being studied—in which clarifying results could be obtained if the technique of studying "pure" substrains obtained by inoculation of limiting dilutions were applied.

As far as can be judged from a fairly comprehensive practical experience in the fields of bacterial and virus variation and from a survey of the literature, there seems to be no valid evidence against the working hypothesis:

That heritable variations in bacteria and viruses arise by a process of discontinuous mutation essentially similar to gene mutation in higher forms and that the mass transformation of a strain as observed in practice is the result of selective survival and overgrowth of one or more mutant types.

This is the point of view which will be adopted throughout these lectures.

As yet there is very little evidence as to the nature of the mechanism in bacteria and viruses which is responsible for this appearance of discontinuous mutants. Avery's recent work (14) on the substance which mediates the reversion of rough pneumococci to a heterologous smooth type, however, opens up an important new approach and provides significant evidence of the essential continuity of genetic processes throughout living organisms. Without going into details, his experiments indicate that amongst the nucleic acid molecules which can be extracted from smooth pneumococci (type III) are some which can be taken up by rough derivatives of type II which have an inherited inability to produce type-specific polysaccharide. The nucleic acid molecules so taken up appear to be incorporated into the genetic mechanism and confer an inheritable capacity on the organism to produce type III polysaccharide. Subject to modification in the light of future work, the only straightforward interpretation of the phenomena is that the S–R mutation is a result of the loss of a certain type of nucleic acid molecule from the controlling genetic mechanism. The obvious corollary is that this genetic mechanism is like the chromosomes of the higher forms essentially of nucleoprotein structure, and that the "genes" or equivalent units have their specific activity determined by the chemical pattern (cf. the immunologically relevant determinant groups of a protein molecule) of their nucleic acid constituents. The change of type is induced by the filling of the gap in the mechanism with an appropriate but specifically distinct nucleic acid unit.

Huxley (6) in discussing the application of genetic concepts to bacteria says:

> Bacteria have no genes in the sense of accurately quantized portions of hereditary substance . . . the entire organism appears to function both as soma and germ plasm and evolution must be a matter of alteration in the reaction system as a whole . . . there is no ground for supposing that mutations are similar in nature to those of higher organisms. One guess may be hazarded that the specificity of their constitution is maintained by a purely chemical equilibrium without any of the mechanical control superposed by the mitotic and meiotic arrangements of higher forms.

The work on bacterial variation which we have discussed above suggests that this attitude is merely a reflection of the inadequacy of available knowledge. One would hazard in fact the prophecy that when "academic" research is again permissible the study of micro-organismal genetics *will* show that bacterial hereditary mechanisms are accurately quantized and in addition provide data of fundamental importance for the interpretation of organic evolution. Beadle's work (15) on the genetic control of the chemical processes in the fungus *Neurospora* is outside of the present theme, but it provides an exciting forecast of what future studies on bacteria and viruses may bring to light. For the present, Huxley's view can be legitimately applied to the smaller viruses; in these the entire organism does seem to function both as soma and germ plasm.

Although they are not as yet susceptible to quantitative analysis, there are two features of variation in viruses which are of considerable interest in their relationship to variation in higher organisms. The first is that when mutation occurs the change will normally be to one of a small number of new configurations which appear to be more genetically stable than any of the alternatives. One may mention, as examples, the change from smallpox virus to vaccinia, street virus to fixed virus of rabies, and the O–D change in influenza virus A.

The second feature which can be observed in work with influenza virus and is probably of general application concerns progressive change in character. When a mutant has arisen and by selective survival become the predominant form, a new "stepping-off place" is established, allowing mutants to appear which would not be obtainable direct from the parent form. The evolution of pathogenic micro-organisms as of higher forms must be based very largely on this characteristic, that with each change in heritable structure new potentialities of further change open out.

SURVIVAL

Pathogenic micro-organisms in general and viruses in particular survive under conditions which contrast vividly with those of most higher types of organism. Consider as an example influenza virus A. A small number of virus particles reach the respiratory epithelium of a susceptible person, and an infection is initiated. Within two days a population of many billions of particles is present in or on the respiratory epithelium. Of these all but an infinitesimal proportion die without descendants. A few may induce infection in another susceptible person. Eventually the epidemic disappears, and as far as present knowledge goes the immense temporary populations of virus have all been destroyed. Perhaps in one person in 10,000 a few virus particles remain latent from which under suitable conditions a new epidemic may be initiated.

The details of the process vary enormously from one pathogen to another, but it is a general feature to find an extremely rapid build-up of isolated substrains of the species to a very high population level, followed by the extermination of the vast bulk of the population and the regeneration of the strain from a very small number of individuals, possibly only from one. Although the scale of time and space is very different, the process is biologically not very dissimilar to the periodic over-population and catastrophic destruction of lemmings and other small rodents, or the process by which enormous numbers of winged termites perish for every pair that found a new colony.

These characteristics of pathogenic bacteria and viruses have some interesting implications when looked at from the standpoint that an ecologist would use to view the distribution and evolution of some species of bird or mammal.

If the occurrence of any possible mutation is, as we presume, a function of the number of individuals in the population, the amount of variance provided by mutation (on which selective survival may work) will vary directly with the size of the population. During a period of epidemic prevalence there will, therefore, be an abundant supply of all the variants that the initially infecting strain can provide. No variant, however, will replace the original unless it has some definite short-period survival advantage, and in any case its chance of doing so will be rendered small by the minute proportion of the population in any infected host which find any opportunity of survival. If, for example, a variant with some considerable survival advantage appears approximately once in 10^6 generations, and that not more than $1/10^7$ of the virus particles in a given host will find an opportunity of initiating further infection; then, of say 10^{10} particles present in the host, about 10^5 (allowing a 10-fold advantage to the variant) will consist of the new form. There will, therefore, be in the infecting dose for the new host 10^8 original type particles and a 1:100 chance only of the variant being present, despite its survival advantage.

Once a variant of high pathogenicity, i.e., with a short-term survival advantage, succeeds in becoming the dominant form in a susceptible host, it may be able to spread rapidly through what susceptible host population is available, giving rise to a subepidemic of unusual virulence. Survival of the species from one epidemic to another will depend on a relatively very small number of individuals, and there will, for this purpose, tend to be a survival advantage for variants of low rather than high virulence. Again, however, we come up against the very low probability of a suitable mutant being present in the minute fraction of the species "chosen" by chance to continue in existence during inter-epidemic periods.

For all these reasons it is impossible to give any general rules in regard to variation in viruses (or other pathogens) under natural conditions. At least we can say that what is commonly observed, namely, a general uniformity in the behaviour of the disease from year to year with minor prevalences of unusually high or unusually low virulence, is compatible with what would be deduced from such a discussion.

II

EVOLUTION AND CHANGE IN VIRUS DISEASE

THERE are three main possibilities to be considered in any speculations as to the origin of the viruses and their relation to other forms of life. The first is the hypothesis that some viruses took their origin as pathologically active fragments from the cells of higher forms. A common way of expressing this conception is to compare it on the cellular level with malignant change. A cancer cell is a cell derived like every other cell in the body from the fertilized ovum, but it has an independent unco-ordinated proliferative activity of its own and behaves very much like a parasitic protozoon. In some rare instances, e.g., the genital sarcoma of dogs mentioned by DeMonbreun and Goodpasture (16), there is even a possibility that the malignant strain of cells can be implanted by natural means in another host. It is conceivable that a somewhat similar escape from normal restraint might involve part of the genetic system of a cell—a gene multiplying abnormally within the cell might well produce effects essentially similar to those of a virus. Once produced, such a foot-loose gene might perhaps find it possible to survive by transfer from host to host in the fashion of a virus. There is no evidence that any such process occurs. Some of the viruses at least, e.g., psittacosis and vaccinia viruses, are certainly not derivatives of vertebrate cells, and for the great majority of viruses the possibility can be discarded. The very strict species and tissue specificity of the mammalian papilloma viruses perhaps gives a hint that this particular group might have had its origin in the last analysis from host cells. It is, however, equally possible that these viruses have only developed their high specificity as a secondary characteristic and are ultimately of independent extrinsic origin. Shope's rabbit papilloma in its natural state as an affection of the jackrabbit in Texas seems to spread naturally in the normal fashion of an infectious disease.

The second possibility is that viruses represent the surviving descendants of primitive precellular forms of life. When the dominance of higher forms was established they failed to survive, except in the protected environment given by the adoption of a strictly parasitic mode of life. The only available evidence in support of such a view is the existence of such filterable saprophytes as those described by Laidlaw and Elford (17). It is probable that this group may be related to the parasites and semiparasites of the so-called pleuropneumonia-like organisms, and Sabin (18) has combined all these into a new class, *Paramycetes*. It is quite unlikely, however, that they have any close relationship to the viruses proper.

The third and at the present time most widely accepted view of the origin of the viruses is that put forward by Green and by Laidlaw. For them, viruses represent the degenerate descendants of larger pathogenic micro-organisms. Probably the most cogent argument for such an origin is the existence of an almost continuous range of forms between typical bacterial pathogens, like the streptococcus or the typhoid bacillus, and the smallest viruses.

The rickettsias have almost certainly evolved from the bacterial symbionts present in many types of arthropod (19). They have the appearance and staining qualities of small bacteria, but for their growth demand some component or enzyme system that it has not yet been possible to incorporate in a non-living medium.

The viruses of the psittacosis group are relatively large, the standard forms being about 0.3μ in diameter. They stain like bacteria, and their pathogenic activity is on the whole more like that of a moderately virulent bacterium than a typical virus. Some are susceptible to the therapeutic influence of sulphonamides. Their requirements for multiplication are, however, those of a typical virus. In tissue cultures they multiply predominantly during the period over which the cells remain viable, in contrast to rickettsias which multiply best when the cells have passed the stage of maximal vitality.

The medium-sized viruses, of which vaccinia is the type, are relatively complex and might also be derived from bacterial ancestors. The smaller viruses have so few characterizing features that if they are derived by parasitic degeneration from larger forms these might just as well be protozoal, spirochetal, or fungal in character as bacterial. There is no reason why parasitic degeneration should not occur equally often in these groups. Indications of such degenerative processes may be seen in *Anaplasma* amongst the protozoa and the granular forms of *Spirochaeta recurrentis.*

A great deal of light has been thrown on the process by which parasitic degeneration in micro-organisms takes place by the work of Beadle and his collaborators (15, 20) on the mould, *Neurospora.* In its natural form *Neurospora* has very simple nutritive requirements: a simple carbon source like glucose, nitrate or nitrite to supply nitrogen, inorganic salts, and one vitamin, biotin. Variants can be produced by X-radiation which fail to grow on a simple basic medium of this composition but will grow on a "complete" medium containing a wide range of amino acids and vitamins. Biochemical and genetic analysis of more than 200 such variants has shown, first, that the mould in the course of its growth synthesizes all the major chemical components—amino acids, B-group vitamins, etc.—of living tissue generally. Secondly, each variant is found to lack the capacity to synthesize *one* essential nutrient or metabolite; and thirdly, genetic analysis shows that in each case the change is the result of a typical gene mutation.

Practically all these changes must result from damage to some portion of the genetic apparatus—what was an orderly molecular arrangement with meaning and function has been distorted to non-function or actually lost. Similar changes in other primitive organisms, fungi and bacteria, as well as in *Neurospora,* are probably forever occurring spontaneously. If they result in diminished efficiency of the organism, the mutant strain will fail to survive; but where the loss is of an ability to synthesize some nutrient constantly present in the environment, the mutation may have no effect whatever on the organism's chance for survival.

If, for example, a saprophytic bacterium capable of synthesizing its structure from the simplest nutrients finds a habitat in the intestine of a vertebrate it will have immediately available many of the intermediate substances which it would otherwise need to synthesize. Any mutation to a form lacking the ability to synthesize one of these available nutrients will suffer no disability in this environment. If the organism takes a further step towards parasitism and invades the tissues, it will find a still richer source of biological molecules and can, if need be, do without very many of the synthetic abilities of its free-living ancestor.

The evolution of the viruses can then be regarded as resulting from a series of mutations by loss of power of chemical synthesis associated with progressive adaptation to an environment (the intracellular one) in which such ability is not required for survival. The existent viruses represent various stages on this path, the theoretical limit of which is reached when we have a bare nucleoprotein molecule capable of self-replication but drawing the necessary material and energetic systems wholly from the metabolic activities of the host cell. The smaller viruses appear to represent or approach this limit.

It must not be forgotten that an evolution of this sort could not progress by negative changes alone. To survive as a species, the parasitic micro-organism must at every stage of its evolution find a means of ensuring transfer to fresh susceptible cells and hosts. In general this will mean that in a significant proportion of invaded cells necrosis and reliberation of the pathogen must occur. As to the nature of the adaptations developed to ensure entry into a susceptible cell and subsequent liberation of descendant units, we have at present only slender clues. Hirst's discovery (21) that influenza virus is adsorbed specifically to various types of vertebrate red cells and after a certain lapse of time reliberated gives some hope of an effective approach to the problem. The phenomenon of "interference" between virus strains referred to later (p. 38) may provide other clues. It is in this field that one may expect in the next few years to see the major advances in the more theoretical aspects of virus research.

If we accept the thesis that viruses are the degenerate descendants of larger pathogenic micro-organisms—and there seems to be no visible alternative—a long evolutionary history must be postulated for them. To attempt an account of this history requires an effort of imagination comparable to that which would be needed to describe the evolution of the vertebrates in the complete absence of palaeontological data. In place of comparative morphology and embryology, we have to work on the physio-chemical characters of existent viruses and other pathogenic micro-organisms, and on their host range, mode of spread, and similar ecological attributes.

No one would consider that a series of existent vertebrates from amphioxus through lamprey, shark, lung-fish, frog, lizard, tree shrew, and monkey to man gives an adequate picture of human evolution. But, in all probability, it provides a useful picture of the general type of each of the main stages. It is impossible to give even as precise a series as this for the ancestry of any given virus, but we can suggest at least two series of forms which may parallel fairly closely the principal lines along which viruses have been evolved.

The first series is of micro-organisms transmitted by insect vectors from other mammalian hosts, mainly rodents, to man. It includes the organisms responsible for bubonic plague, tularaemia, oroya fever (*Bartonella bacilliformis* infection), murine typhus, and yellow fever. In this series we have a general diminution in size, although in this respect the gap between the typhus rickettsia and the virus of yellow fever is much greater than that between any of the others. There is a fairly regular decrease in the synthetic ability of the organisms. The plague bacillus grows readily on standard bacteriological media; *B. tularense* and the bartonellas require special media; while rickettsias and viruses will grow only in suitable living cells. As Zinsser pointed out, there is a characteristic difference between the behaviour of rickettsias and viruses in tissue culture in that the viruses multiply only while the cells are actively living, whereas rickettsial multiplication goes on and is often maximal at a period when the cells are no longer viable.

For a second series we might take a range of infections spread without intermediate vectors and mainly involving mucous membranes—streptococcal infection, psittacosis, smallpox, influenza. Only the first of these is due to micro-organisms which can be cultivated on non-living media, but the psittacosis virus belongs to the group of agents which are clearly, in some sense, intermediate between bacteria and more typical viruses—in size, in staining qualities, in the type of lesion produced, and in being partially susceptible to sulphonamides (though psittacosis virus itself is an exception to the last). The smallpox–vaccinia virus is a typical enough virus, but it is larger than influenza virus and in electromicrographs shows evidence of internal structure. Chemically, too, it appears to be considerably more complex and approaches some simple bacterium in constitution.

It is quite obvious that the plague bacillus is not an ancestor of the typhus rickettsia, or the streptococcus of vaccinia virus, but at least it is easy to see that somewhat similar pathogenic bacteria in some warm-blooded host may have been the ancestral forms. From the present-day distribution of pathogenic organisms it is clear that we cannot think of viruses or rickettsias as having necessarily developed in parallel with the hosts they now parasitize. If we accept the normal evolutionary sequence as free-living saprophytic micro-organism (bacterium, protozoon, or fungus)–faculative parasite–obligatory parasite with subsequent degenerative specialization to virus, we must expect to find more than one change of host in the course of the story of any given virus.

Like any other species of living organism, a virus now existent is the current manifestation of an ability to survive. It has been able at every stage of its evolution to find conditions for multiplication and to ensure that at all times descendant organisms were being produced—not necessarily in the numbers needed to replace those destroyed, but sufficient to allow the continual regeneration of the species. In talking of viruses (or of pathogens in general) this thread of survival must always be a central theme. In fact, even a theologian might be at a loss to see anything more than multiplication and survival reduced to its lowest possible terms when we deal with a virus like that of infantile paralysis. If we could follow its evolutionary history there would undoubtedly be many surprises in store for us. It would be a story with phases of rapid multiplication and equally rapid cataclysmic destructions, of dissemination of descendants to every variety of new biological environment with everywhere the test of survival being applied. With each new or modified environment in which continued survival was possible, mutation and selection would see to it that a gradual adaptation would provide characters tending to make survival more assured. And with every adaptive change some other new potentiality of establishing itself in another environment might arise. There is more than a likelihood that some human pathogens evolved primarily as saprophytes and later parasites of the alimentary tract of insects, pathogenicity for man developing only accidentally in association perhaps with adaptation to survival in a blood-sucking insect. The accidental is always a possibility in the evolution of the viruses, if by accidental we mean such faculties of the organism as have not been developed as adaptations to favour its current mode of survival.

It is only possible to come to closer terms with the problem of virus evolution if we restrict our attention to those types of change in disease character or host change that we can see now in progress. This means that we have to take for granted all the stages by which the saprophytic ancestor became the strictly parasitic virus and concern ourselves mainly with the implications of change in host species and particularly of transfer of virus infection from animal hosts to man.

THE HOST–PARASITE RELATIONSHIP

It was once easy to think of the interaction between man and some pathogenic micro-organism as a simple antagonism, success for the pathogen being measured by its own overwhelming multiplication with death of the host, success for the patient by recovery with annihilation of the invaders. But once the pathogen began to be thought of as a living organism dependent for its existence on a means of continuous survival, such a point of view became obviously untenable. Success for a parasite as for any other living organism is measurable only by the size and persistence of the population, the number of individuals which make up the species. It is irrelevent to that success whether infection is fatal to every host individual or to none. But it is relevant that, with only an occasional exception (e.g., anthrax), once an animal has died of micro-organismal infection there is no further possibility of the pathogen being transferred to new hosts except in very special circumstances. An acutely fatal infection is, therefore, disadvantageous for the survival of the parasite. Conversely, a low-grade infection with no more than trivial symptoms but with free liberation of the pathogen over a considerable period of time will usually provide the maximum opportunity for dissemination of the parasite. With many sorts of qualification and limitation the normal end result of long-period interaction under approximately constant conditions between host and parasite is a state in which the host suffers no significant disability and the parasite persists long enough to ensure transfer by one or other method to a new susceptible host.

Such an equilibrium resembles many other balanced interactions between living species (e.g., between carnivores and their herbivorous prey), and like most of them is rather an ideal conception than a commonly demonstrable fact. The most usual finding is an oscillation of variable period and intensity in the balance between predator and food species or between host and parasite; in the latter case, this takes the form of periods of increased or decreased prevalences of infection. These oscillations may be determined by a variety of factors—in part environmental, in part associated with biological variation in parasite or host. In the particular field of virus disease, variation in the parasite will normally be mutational; variation in the host may also be of genetic origin, but it is much more commonly due to immunological change.

It is an interesting speculation as to how the possibility of immunological variation arose in the course of evolution. In practically every other type of interaction between two species, if the environment remains constant, change in the status quo is possible only through the appearance of a genetically determined variant of one or other species and its progressive replacement of the original form by a process of selective survival. If this were the only way by which new contacts between mammalian hosts and pathogenic micro-organisms could be resolved, it would provide an intolerable advantage to the micro-organism, with its immensely greater numbers and shorter generation time. The development of an alternative mechanism to counter micro-organismal attack can be regarded biologically as one of the first necessities to be acquired as animals grew larger and generation times longer. It is possible that amongst the small rodents which suffer violent population changes of short period, genetically modified resistance to infection may play some part in the interaction with pathogenic micro-organisms; but even with these, immunological changes in resistance occur. In all higher mammals, resistance to infection is essentially an individual matter only very indirectly related to the genetic make-up of the species.

So we have the standard picture of recurrent epidemics of virus disease whenever a sufficient density of susceptible subjects arises, either by entry into the community through birth or from an uninfected region or by the waning of previously acquired immunity. The details of this process will vary greatly from one type of infection to another; immunity is notoriously effective against one type of virus and almost without visible effect against another. When only clinical and epidemiological facts are available, the apparent irregularities in behaviour may be much greater than they would seem if knowledge were available of the real extent of infection. In virus disease, as in other types of infectious disease, the standard mode by which the parasite survives may not necessarily be that by which it induces symptoms of disease in the host. Infantile paralysis is almost certainly a mere accidental associate of infection by the virus of poliomyelitis, but technical difficulties make it impossible to assert this with the certainty that attaches to some of the bacterial infections. Cerebrospinal meningitis can be taken as a convenient example. The meningococcus was first isolated as the cause of cerebrospinal meningitis, and almost its only practical importance in medicine is as the agent of that disease. But this capacity to invade the blood and the meninges of certain susceptible individuals plays no part in furthering the survival of the meningococcus as a species. Its real life is carried on as an almost saprophytic inhabitant of the human throat, from which it is transferred to other individuals by the usual modes of respiratory spread. Only by the coincidence of an unduly susceptible host and an invasive variant of the parasite can the characteristic clinical disease develop. Probably without exception, every strain of meningococcus isolated from the cerebrospinal fluids of typical cases is descended from organisms which never in their whole evolutionary history have produced meningeal infections. From the point of view of the meningococcus, its power to provoke meningitis is a mere biological accident, of no significance for its survival or evolution.

Whatever visible character it may take, the development of a more or less balanced interaction between the two species—host and pathogenic (or potentially pathogenic) micro-organism—requires (1) a prolonged period of association between the two, (2) opportunity for infection to involve a high proportion of the individuals of the host species, and (3) the absence of any important means by which the pathogen can survive indefinitely in the absence of the host. When these conditions are fulfilled, we may expect to find as standard a low-grade, widely prevalent infection only exceptionally producing significant mortality in the host.

Amongst human virus infections which seem to fulfil these criteria we can distinguish several epidemiologically distinct groups. First, we have the specific infective fevers—measles, rubella, chickenpox, and mumps—in which the standard picture is a mild infection during childhood followed by lifelong immunity at least to symptomatic reinfection. In a second group are influenza and the viruses presumed responsible for the less clearly defined respiratory infections, while the third and last group contains only herpes simplex. None of these are diseases which can be regarded as serious for the human species as a whole or for any large community. The respiratory viruses are probably responsible for the final illness of many old people, but this, biologically speaking, is of no importance whatever to the functioning of the species.

When conditions 1 and 2 are not completely fulfilled, happenings are liable, epidemiologically, to be more striking and often disturbing. An interesting recent occurrence in Australia concerns rubella. There is no obligation to report German measles in any Australian state, and no figures of its prevalence are available, but it is believed on substantial evidence that this infection was absent or at least never developed a capacity for epidemic spread during the twenty to twenty-five years prior to 1937. From 1937 to 1942 it was widely prevalent, and a very large proportion of the young adult population suffered an attack. Most cases were mild, but many, particularly amongst those seen in military camps, were more severe than the traditional case of German measles. The feature of greatest interest, however, has been the occurrence of congenital abnormalities in the children of women suffering an attack of rubella in the first or second month of pregnancy (22). This may represent the development of a new capacity on the part of the virus, but more probably it is only a reflex of the unusual opportunity presented to the virus by the existence of a large young adult population susceptible to infection.

The history of smallpox in Europe provides a somewhat similar picture of a disease which apparently never succeeded in developing a full endemic status. In the sixteenth and seventeenth centuries in England it was a relatively rare disease, at first most frequent in adults. Its incidence increased and tended to involve progressively younger age groups till in the eighteenth century it had become almost wholly a disease of childhood. It is difficult to judge what the mortality of the disease was at this period. That considering the hygiene conditions of the times it was not excessively high is indicated by the fact that when for some reason (partly the introduction of vaccination) smallpox rapidly diminished in importance at the end of the eighteenth century, measles took its place as the biggest cause of childhood mortality and the total death rate was hardly altered. Smallpox apparently never develops a capacity to spread with the vigour of measles or chickenpox, and concentrated epidemics occur only under unusually favourable conditions for spread of the virus. Australia has been fairly often invaded by smallpox but despite a very poorly vaccinated population it has never experienced a serious epidemic.

The second group of human virus diseases which fulfil the three criteria for the development of an equilibrium state are the localized infections of the respiratory tract. Here there is no lasting immunity following infection, and the equilibrium condition appears to depend on a constant persistence of the viruses in the community, with recurrent and usually subclinical infection. The different members of the group probably vary considerably in the length of the interval between recurrent infections, epidemic influenza providing a considerably more definite post-infectious period of immunity and forming in a sense a connecting link between the first and second groups.

Simple herpes is apparently unique amongst human diseases in being able to persist from generation to generation without importation from outside. It is the only known virus disease in which a child can become infected from its parent and remain infected long enough to transmit infection to its own offspring. This is a characteristic found in certain animal infections, notably lymphocytic choriomeningitis and Theiler's disease in mice and psittacosis in parrots and other birds, and allows the possibility of a rather different evolutionary history for the viruses concerned.

Herpes simplex may well be a virus which has evolved with man right through his mammalian history. The detailed discussion of this hypothesis can be left to a subsequent chapter. Here it is only necessary to point out that herpes in this respect offers sharp contrast to the other human virus diseases.

It is generally considered that in the early stages of human evolution primitive man and his subhuman progenitors existed in small wandering groups of at most a few families, and that these groups only rarely came into contact one with the other. Under such circumstances it would be virtually impossible for a pathogen to evolve as a specifically human parasite unless, as is the case with herpes simplex, the period over which a person remained capable of transferring infection was of the order of a generation. For other types of virus disease to fulfil the three criteria which have been given as necessary if a host–parasite relationship is to reach what is equivalent to an ecological climax condition, it is essential that the host species should be composed of large aggregates of individuals and that these aggregates should interpenetrate in an epidemiological sense, i.e., sufficiently to allow transfer of parasite strains from one aggregate to another. In other words, the evolution of virus diseases will in general take place in gregarious species. Species which are composed of solitary individuals or of small groups only will be subject to casual infection from such viruses of other (gregarious) species as they may encounter. These infections will initially at least be mere accidents of no biological significance for the survival of either host or parasite. But if an animal species increases greatly in numbers, or changes its habits toward a more gregarious mode of living, new opportunities for parasitic evolution may open out following casual infection. It is by the exploitation of such opportunities that most of the viruses now pathogenic for man must have arisen.

In most instances, such entry of virus into the cells of an alien host will provoke nothing but a trivial infection overcome without symptoms and without any possibility of transfer to individuals of like species. At times, however, it may so happen that the intruding potential host is highly susceptible to the virus. A good example from experimental work is the use of the Java sparrow to detect the presence of latent psittacosis in a budgerigar aviary (23). It is impossible to say why a virus like psittacosis is highly virulent for one species, like the Java sparrow, and hardly at all for another, like the domestic fowl. But the irregularity of virulence for different species is a characteristic of experimental work on viruses and must be accepted as a fact. Further, there is also no doubt that at times virus variants arise or are produced which have an abnormally high virulence for some alien host. Whatever the reason, it may happen in nature that a virus transferred to a new host can flourish and, with or without death of the host, be capable of transferring from individual to individual of the new host species. If the new host is a solitary species the infection will go no further than the individual, but if it is gregarious a new ecological conflict arises which must eventually be resolved either by the elimination of the virus, the extinction of the host species, or the development of a new climax state. Such a process of irregular transfer from one dominant gregarious species to another probably represents the standard evolutionary history of virus diseases, or indeed of any type of strictly parasitic organism.

To return to the question of the specifically human virus diseases: we have given reasons for believing that in the early phase of human existence, from the beginning of the Pleistocene up to about 10,000 years ago, infectious disease due to micro-organisms specifically adapted to the human species was almost nonexistent. The herpes virus could have persisted with very much its present type of activity, but the viruses producing brief infection with subsequent immunity—measles, mumps, and the like—could obviously not have survived in anything like their present form. Only when agriculture began to replace food-gathering and larger settled communities took the place of small wandering bands could accidentally acquired virus infections find an opportunity for extensive transfer to other individuals and for their development to specifically human diseases.

The first stage in the development of an endemic virus disease of man would, in general, be the transfer to the human host of a virus that had evolved as a specific parasite of some other abundant species. If the virus was of sufficient intrinsic virulence for man and found the necessary circumstances to provide means for its spread through the population, it was enabled to survive as a human parasite and to begin a new evolution toward a persisting dynamic equilibrium with its host.

Such a conception will be more readily accepted if we think of the "new" human diseases which have appeared and been adequately studied in the last fifty years: psittacosis, St. Louis encephalitis, western equine encephalomyelitis, paralytic rabies in Trinidad, Q fever in Queensland and the western United States. All are apparently diseases of mammals or birds which have either developed new virulence or found new opportunities of transfer to man.

Psittacosis is of particular interest, since a number of recent episodes suggest that it has the potentiality of developing into a specifically human disease. Person-to-person infection is apparently by no means as rare as was formerly thought.

Of older diseases which seem to have taken on their standard human form within the historic period, yellow fever and epidemic typhus may be mentioned. Yellow fever will be discussed in a separate chapter.

Epidemic typhus, as it is seen in eastern Europe or North Africa, is a fully established human infection, but there is fairly strong historical evidence that the typical disease has been known in Europe only since the fifteenth century. Zinsser's assumption (24) that the typhus rickettsia is an ancient parasite of the rat and its fleas, and that the murine form is ancestral to the classical type spread from man to man by the louse, is fairly generally accepted. But in these rickettsial diseases we have a complex evolutionary problem in which the part played by the arthropod vector dominates the picture. There are good reasons for believing that the rickettsias are organisms which developed originally as saprophytes or semiparasites in the gut of the arthropod and were transmitted to the next generation through the egg. The power to infect the mammalian hosts on which blood-sucking arthropods fed was of survival value to the rickettsias primarily by allowing transfer from one strain of arthropod to another. A further factor may have been some correlation between the power to multiply freely in the blood-engorged intestine of the arthropod and the power to infect the mammal which provided the blood, but no evidence for such a correlation is yet available. On the whole, rickettsias are without special preferences in regard to their vertebrate hosts; they will infect most mammalian species that have been tested, in most cases with only trivial symptoms. Man is of all known hosts probably the most generally susceptible. In nature it is usual to find that all the small animals which serve as feeding grounds for the ticks, mites, or fleas that carry pathogenic rickettsias give a proportion showing evidence of infection.

Endemic or murine typhus is nowadays usually contracted from rats via the rat flea but there is at least one epidemic on record (Toowoomba, Queensland, 1925) in which the source was almost certainly to be found in mice (25). It has been shown that in areas where endemic typhus is common and there is a human population heavily infected with lice, small epidemics of *louse-borne* infection can be initiated from an individual infected with the murine type of typhus rickettsia. Zinsser and others hold that epidemic typhus, the great scourge of armies, prisons, and the destitute from the sixteenth century onward, arose out of such episodes, perhaps in Hungary during the fifteenth century. At the present time active work is in progress on the experimental differentiation between the rickettsias of murine and epidemic typhus. The differences may be greater than was once thought, but they are no more than might be expected to arise between collateral descendants of the same ancestral type transferred for centuries in two different biological environments. Any suggestion that typhus is really an ancient louse–man disease is rendered extremely unlikely by the fact that even now the body louse infected with typhus rickettsias inevitably dies of the infection. The rat flea, on the other hand, becomes a strong candidate for the original vector in virtue of the fact that it can be heavily infected without suffering any apparent inconvenience.

The real difficulty of the hypothesis that all or most human epidemic diseases are derived from diseases of animals which had earlier lived in (epidemiologically speaking) crowded groups concerns the specifically human virus diseases which can only be transmitted with difficulty, or not at all, to any of the available laboratory animals and which have no known relatives in the animal sphere. They include measles, rubella, mumps, chickenpox, molluscum contagiosum, and warts. To take measles as an example: this can be transmitted in a rather mild and atypical form to monkeys by inoculation; it is not contagious from monkey to monkey nor is there any other known susceptible species. The only animal diseases with which it has anything in common are the acute generalised infections like canine distemper, swine fever, and rinderpest. All of these are transmissible by the respiratory route, have a moderately long incubation period, and show an acute invasion of the blood stream by the virus in the early febrile period. Like measles virus, none of these viruses are transmissible to zoologically unrelated species and none show any unequivocal ability to infect chick embryo tissues. Measles produces no demonstrable lesions in this situation although with some strains at least multiplication undoubtedly occurs.

Epidemiologically it is obvious that measles could only persist in large communities and could not have been present in anything like its present form amongst people in the food-gathering stage of development. As a virus disease it must, therefore, be derived from some other species, and we are driven to conclude that since it became a human disease the virus has entirely lost its pathogenicity for the original host, whatever species that may have been. Since neither measles nor the animal viruses mentioned above can be adequately studied in small laboratory rodents or in chick embryos, and work with the natural host has for economic reasons been almost wholly occupied with the problem of prevention and immunization, there is no information available for discussion of possible relationships.

III

THE REACTION OF THE HOST TO VIRUS INFECTION

THE INTERACTION of virus and host, which as a norm results in the continued survival of both species, is a dynamic process carried out in a constantly changing environment. To maintain or re-establish such an equilibrium may require active modification of either organism. The potentialities and limitations of the virus to meet changing host situations have been discussed in the preceding sections, the view being adopted that effective adaptation is only possible if, from mutations spontaneously occurring, a modified form with the required properties can survive preferentially until it replaces the original as the dominant form. The host species is not so limited in the modes by which it can counter the attack of the pathogen. Genetic factors undoubtedly play a limited role in determining the susceptibility or resistance of a mammalian host to a given infection, and particularly in small rapidly multiplying rodents the elimination of susceptible and the survival of genetically resistant strains may be an important part of the ecological process. There is very little evidence that inheritable variations in resistance play any part in relation to virus disease in man. The relative insusceptibility of Negroes to yellow fever is the only example where a reasonably good case can be made for the existence of inherent racial differences in resistance to a virus infection. Even in this case it seems highly unlikely that the resistance of the Negro is due directly to the appearance and selective survival of appropriate human strains, in an environment heavily infected with the virus. More likely the racial resistance is a character accidentally associated with some other genetic factor.

Except for these minor possibilities of genetically determined changes in resistance of species or race, the defence of the host depends wholly on individual physiological activities, and increase in the effectiveness of resistance is nearly always of immunological origin. Both the traditional lore and the early scientific study of immunity centred on the dramatic change in the susceptibility of an individual that followed an attack of some of the easily recognised virus diseases. It was common knowledge that until a person had taken the smallpox he remained susceptible to the disease, but once he had so suffered and recovered he was immune for life. Closer study of immunity to virus disease has shown that long lasting immunity follows only a limited number of types of infection. Some show an almost equally striking lack of specific immunity, and even when immune reactions seem to dominate the picture of individual resistance to infection other factors need to be considered as well.

The internal situation in an animal invaded by a virus which it has not previously encountered is probably more complex than is usually thought. Very little is known as to what determines cellular susceptibility to effective invasion by a virus. One can broadly regard viruses as restricted to one or other system of tissues in the body, as being epitheliotropic, pneumotropic, neurotropic, and so on. Actually these limitations are rarely absolute and can nearly always be overstepped by strains of abnormally high virulence in either natural or experimental hosts. Within a given system of tissues there are usually certain types of cell which are more susceptible than others. In the central nervous system, poliomyelitis virus selectively attacks cells of the motor system. Influenza virus in monkeys, and probably in man, attacks the bronchiolar epithelium more severely than any other part of the respiratory lining.

The virus strains used in experimental work are normally maintained at maximal virulence, and the pathological changes produced probably give an erroneous impression of the regularity of attack on all exposed cells of the susceptible system of tissues. In natural infections of lower intensity there is often a very patchy attack on the susceptible tissue. This can be seen in experimental poliomyelitis both in the rhesus monkey and in the chimpanzee, and must certainly be the case in the numerous instances of non-paralytic poliomyelitis in man where invasion of the central nervous system has undoubtedly occurred but no demonstrable paralysis has resulted. There is evidence so far from only a very limited number of experimental studies that virus may make a type of contact with a susceptible cell which does not result in multiplication and necrosis of the cell but renders it insusceptible to the attack of a more virulent strain of the same general character. "Interference" or blockade phenomena of this type have been described for yellow fever (26, 27), influenza (28), and equine encephalomyelitis viruses (29). What appear to be similar phenomena have also been reported for plant and bacterial viruses. No detailed interpretation of these reactions is yet possible, but it seems certain that they will eventually be found to be of great importance in determining the character of the subclinical and abortive infections which make up such a large part of human experience of virus disease.

Some infections by some viruses can undoubtedly be overcome very rapidly by hosts that have had no previous experience of the infection. Unless some quite unexpectedly rapid local immunological mechanism is brought into play, resistance in these instances is developed quite independently of antibody production. Even in the chick embryo, which has no capacity whatever to produce antibody, many virus infections of the chorio-allantois are rapidly dealt with and the initial lesion cast off. Interference phenomena may well have an important role in these examples of rapid effective resistance. Another mechanism associated with the rapid acquisition of resistance to virus attack before the immune response is effective is exemplified by the findings of Francis and Stuart-Harris (30) on influenzal infections of the ferret. Here the respiratory epithelium of the nasal cavity is very largely destroyed by an active influenza virus within 48 hours of experimental infection. Rapid regeneration from the basal cells occurs, and the intermediate type of epithelium produced is then insusceptible to damage by the virus even before there is appreciable antibody in the blood.

The most important mechanism by which infection is overcome and subsequent resistance maintained is that which centres around the production of specific antibody. In these lectures it would be inappropriate to do more than discuss some of the ways in which antibody production by the host affects the ecological situation and alters the epidemiological manifestations of virus activity.

Broadly speaking there are three different situations which may develop:

(1) Infection may be followed by elimination of the virus and permanent or at least prolonged immunity.

(2) Infection persists with repeated opportunities for liberation of the virus despite active antibody production.

(3) Infection of mucous surfaces is followed by antibody production and immunity of varying duration.

These three categories are not sharply delimited, and intermediate conditions may be recognised, but they provide a useful basis of discussion.

As a general rule, solid and prolonged immunity results only when two conditions are fulfilled. The first requirement is that the infection must at some stage involve dissemination of virus through the bloodstream, providing an effective stimulus to the whole antibody-producing mechanism. The second condition is that when an opportunity for reinfection occurs the virus must be exposed to circulating antibody before it can reach those cells which when specifically damaged by the virus provoke the characteristic symptoms of clinical infection. The best example of an infection fulfilling both requirements is yellow fever. At the height of the illness there is a very high concentration of virus in the blood, as high as 10^{10} infecting doses per cubic centimetre. This provides an intense stimulus to the antibody-producing cells, a stimulus whose effect must persist for many years after the elimination of all virus from the body. If an immune individual is reinfected, the virus must of necessity pass through the blood to reach the liver and any other susceptible tissues and is thus exposed to the maximal effect of circulating antibody.

It is highly probable that the two conditions are also fulfilled for the exanthemata smallpox, measles, rubella, and chickenpox. The nature of the initial infection is unknown. Presumably there is a focal infection somewhere on the mucosal surfaces, but the appearance of symptoms is clearly preceded and accompanied by a general dissemination of the virus in the blood. It is quite likely that a person immune to measles may suffer an initial mucosal infection when re-exposed to the virus, but if his circulating antibody can inactivate virus liberated from the primary focus he will show no recognisable evidence of infection. Until better methods of experimental study are available, it will be impossible to say whether mumps and herpes zoster infections fulfil the first criterion of an extensive blood dissemination, but there seems at least no reason to believe that virus can reach the points of symptomatic infection except through the blood.

The most important apparent exception to the rule is dengue. In this disease there is no doubt that the virus is transmitted by a mosquito and that it is present in the blood during the early febrile period. Why immunity is so transient in comparison with that induced by yellow fever is an outstanding problem to be tackled when suitable methods for the study of dengue have been developed.

In the second group—infections which persist despite the presence of circulating antibody—herpes simplex provides the best example from human pathology. Antibody appears in the blood within two weeks of the primary infection and remains present apparently for life. Local recrudescence of activity and liberation of virus may continue to occur indefinitely uninfluenced by the presence of circulating antibody. It is possible that poliomyelitis infection should be included in the group, but so little is known of the source of the poliomyelitis virus, which can be detected in faeces often for some weeks, that very little can usefully be said on the subject.

The third group of mucosal infections with relatively short-lasting immunity is exemplified by the influenza viruses. Infection is of superficial cells which can be reached by the virus either from the environment or from other infected cells in the host without any necessity for passage through the bloodstream. Other factors than antibody are almost certainly also involved in determining resistance to infection, and it is generally believed that the means by which the observed effect of antibody is mediated is the liberation of a small proportion of antibody in the fluids secreted by the mucous surface (31). Virus is clearly much less likely to be hindered in its activity by such a mechanism than if it had to experience exposure to the full level of blood antibody before reaching susceptible cells.

Where permanent immunity with elimination of virus from the body follows infection, the epidemiological picture of the disease is determined almost wholly by this fact. An acute epidemic involving a high proportion of the susceptibles in the community and followed by a complete disappearance of the disease is the standard manifestation where means for the spread of infection are freely available. The disease—measles, for example—reappears when a sufficient density of susceptible persons is regained by birth or immigration. Where the spread is rendered difficult by administrative measures or natural circumstances a more prolonged epidemic of lower intensity will result.

The part played by immunity in determining the incidence of infection in the other groups of virus diseases is much less obvious. Antibody production undoubtedly must be considered in every case as a factor both in determining individual resistance and in modifying the standard process by which the virus survives. Other factors, however, may be relatively much more important in one or other of these respects. Of the factors which may determine resistance to virus disease either directly or in association with immunological responses, the age of the individual is one of the most interesting and important. Man differs from all other animals in the long period needed before he attains full development; and through infancy, childhood, and adolescence there is a changing character of the response to infection which is responsible for a number of interesting clinical and epidemiological aspects of certain virus diseases. Some of the phenomena which require specific mention in later chapters are the characteristic incidence of primary herpes infection between the ages of one and four years, the changing age incidence of poliomyelitis in the last forty years, and the intensity of the attack of pandemic influenza on the young adult age group.

In any relatively stable community, those viruses which produce a relatively lasting immunity will survive mainly at the expense of the new susceptibles introduced by birth. The processes by which infants and children normally become fitted to life in an environment containing pathogenic and potentially pathogenic micro-organisms still require much elucidation, but some general trends can be recognised.

The reaction to micro-organisms which have not previously been encountered seems to show a characteristic sequence of changes with age. In infancy the reactions of defence are liable to be ineffective, fatal infections may occur with little obvious evidence of inflammatory reaction, and when recovery occurs the immunological response is poor. The major need of the infant is protection from contact with pathogens.

In childhood the effectiveness of response to infection increases, and there is hardly an exception to the rule that any infectious disease met for the first time will show a lower average severity in well-nourished children of ages 6–12 than in any other age group. With some diseases the difference is striking: there is practically no mortality in unprotected European children from yellow fever; clinical psittacosis is virtually unknown in this age group; and in the 1918–19 pandemic of influenza, deaths in childhood were negligible in comparison with the high mortality in young adults. The relatively benign character of infection in the young is also evident in some other species, notably in regard to several protozoal diseases of cattle.

As adolescence passes into young adult life there is, as a rule, an increase in apparent susceptibility to non-endemic infections. This is not invariable, and the evidence is liable to be confused by the greater likelihood of persons in this active period of life to be more exposed to certain infections than older or younger persons. The best known examples are lobar pneumonia and typhoid fever, both of which consistently show a peak of mortality in this age group. The phenomenon was particularly evident in the figures for deaths from the 1918–19 pandemic of influenza; it can be seen in the relative severity of measles and chickenpox when these occur in unprotected young adults. There have even been hints in some recent epidemics of poliomyelitis of an undue incidence at this age.

After middle age the impact of infectious disease again increases in severity, and there are several virus diseases which produce their greatest clinical incidence and highest mortality in the oldest age groups. These are psittacosis—almost all deaths are in persons over 40—and two of the mosquito-borne infections of the central nervous system, western equine encephalomyelitis and St. Louis encephalitis.

This sequence of changes with age holds of course only for infections new to the individual. Where a disease is widely endemic, infection either clinical or subclinical will normally occur in childhood and some degree of acquired immunity will result. Sometimes this immunity is permanent; more often it will be maintained at an effective level by repeated subclinical contact with the virus. With such diseases, almost the whole overt incidence will be in children, and the intrinsic susceptibility of the young adult will only appear under unusual epidemiological circumstances.

Some approach to a theoretical interpretation of these relationships between age and the manifestations of virus disease, as of infectious disease in general, can be made along two lines. Like other biological phenomena they can be considered either from the point of view of the physiological mechanisms involved or from the evolutionist's standpoint as adaptive developments with some function in favouring the survival of the individual or the species.

At the physiological level one can recognise an increasing intensity of reaction to a variety of infectious or toxic processes from infancy to young adult life. The phenomenon can be easily seen in the results obtained with various types of intradermal test. A young infant, irrespective of whether or not maternal antitoxin is present, does not react to the Schick and Dick tests. These are reactions to bacterial toxins, but a somewhat similar type of change can be seen with tests for one or other type of allergic reactivity.

In my laboratory we have recently been studying low allergic reactivity to herpes and influenza viruses (32, 33). When suitable preparations are injected intradermally in persons who have been infected with the corresponding virus, an erythematous reaction reaching its maximal size in 24 to 30 hours is observed. With such tests it is very obvious that children's skins react much less severely than adults. Positive reactions are smaller and larger doses are required to obtain clear-cut reactions; non-specific reactions are almost unknown in children but disconcertingly common in adults.

Other examples of this increasing reactivity to various sorts of damaging influence as the child becomes the young adult could be given, but there seems to be no systematic study of the question available and one can make only speculative suggestions as to its physiological basis. It may be that as the individual reaches maturity the cells contain more of those pharmacologically active substances which induce reactive or inflammatory changes, or that they liberate such substances more readily in response to comparable stimuli. In a few unpublished experiments designed to study this possibility in cats and guinea pigs, my colleague, Trethewie, obtained only slight evidence that the amount of histamine which could be liberated from tissues increased with the age of the animal. The phenomenon may well be an essentially human one, to be elucidated only by studies on human material, studies not necessarily confined to histamine estimations.

When we look at these phenomena from the second (evolutionary) point of view, we are asking, in effect, "What advantage for the survival of the human species could accrue from such physiological characteristics?" It may be of course that these qualities are essentially accidental, with no such adaptive significance. It is not, however, difficult to see that the difference between the reactivity of child and adult might be of real importance in primitive human communities. Here endemic infections would inevitably be contracted in early life, so that specific infectious disease of immunized adults would be too rare for its outcome to be of any evolutionary significance. Survival of the human species must have depended very largely on the ability of children and infants to survive their primary contacts with infectious disease. It is possible, though far from certain, that the higher reactivity of the young adult represents a useful adaptation to allow more effective response to injury and traumatic infection. A mechanism which is useful, or at least harmless, when provoked by a local stimulus may be positively harmful when the stimulus is a general one, such as a specific infectious disease. If the average primitive adult possessed a basic immunity to all the endemic generalized infections, an increased capacity to deal effectively with traumatic mishaps might have significant survival value.

The changes in response to disease seen with older individuals are even less susceptible to any detailed physiological interpretation. For obvious reasons, experimental studies on the immunological and physiological reactions of normal human beings have been made almost exclusively on young adults. Sometimes groups of children have been available for study, but most work has been carried out on students and laboratory associates. Middle-aged and older subjects are not accessible to most laboratory workers. The general impression received is that a combination of increasing experience of infection leading to specific and nonspecific resistance is associated with a steadily diminishing activity of response, which at first is beneficial but leads eventually to an ineffectiveness of response that makes the old, like the infant, susceptible to fatal infection by the common low-grade pathogens.

The actual picture presented by an infectious disease will always be determined by many factors, but for any given disease the three major factors responsible for variation in character with time and place are past experience of infection, the age distribu-.

tion of the community affected, and the virulence of the current strain or strains of pathogen responsible. In the following chapters, several of the important virus diseases of man are discussed primarily from the point of view of the ecological interaction of the two species, virus and man. Throughout, the virus, its mode of survival, its characteristic range of variation, and its possible evolution will be the centre of attention, but for each virus it will be necessary also to consider the two interacting human factors of age and immunity.

IV

HERPES SIMPLEX

SIMPLE HERPES, usually of the lips but occasionally located on some other skin area or on the cornea, probably rivals the common cold as the most frequent virus disease of man. The characteristic blisters on the lip that in many people accompany every cold are familiar to everyone, and all clinicians are aware of the frequent occurrence of herpes in most severe febrile illnesses. The general impression produced by the condition is, however, far from that of a typical infectious disease, and it was a considerable time before the discovery of Grüter in 1912 that a virus was present in the vesicles could be properly interpreted. As recently as 1938, Doerr (4) held that herpes was not a virus infection at all in the ordinary sense but a queer physiological response of the cells concerned to the stimulus of fever and certain types of external irritation. The agent present in the vesicles admittedly behaved precisely like a typical virus when rabbits, mice, or chick embryos were inoculated with it, but in Doerr's view it was in the last analysis a product of human cells, not a descendant of pre-existing virus. This view was very much out of key with the Pasteurian doctrine that all pathogenic microorganisms, like every other form of life, were descendants of similar organisms, but at least it might account for the way in which herpetic lesions appear, obviously without infection from the environment.

A more satisfactory alternative has developed in the last few years to bring herpes into line with other infectious diseases. In brief, this interpretation is that herpes infection normally takes place in childhood, the primary infection often taking the form of aphthous stomatitis, and that the infection persists for life. Most of the time it is entirely latent, but appropriate stimuli call the virus into activity and allow it to be reliberated into the environment.

The biggest step toward establishing this conception was taken by Dodd, Johnston, and Buddingh (34), when they showed that most cases of aphthous stomatitis in young children were due to the virus of herpes. In Melbourne we found it easy to confirm this finding and then went on to a fairly extensive investigation of what may be called the natural history of herpes simplex (35).

It was known from earlier work that a large proportion of individuals gave serum containing antibody which could neutralize herpes virus. In making such tests, the serum was mixed with a suitable amount of virus and the mixture tested by intracerebral or intradermal inoculation in rabbits. We had available a simpler and more accurate means of titrating herpes antibody, using the chorio-allantoic membrane of the chick embryo, and most of the experimental work in our investigations took the form of such antibody titrations (36).

Chorio-allantoic membrane titrations depend on the fact that when a suitable strain of herpes or other virus is used visible focal lesions develop on the membrane 2 to 3 days after it has been inoculated with the virus. Stock herpes virus in the form of an emulsion of infected membranes in nutrient broth would usually produce a conveniently small number of foci at a dilution of 1 in 10,000. From the number of focal lesions counted at this dilution the titre can be at once expressed in infective units per cubic centimetre. When diluted virus was mixed with serum from a person not subject to herpes the count of foci obtained did not differ significantly from the control. With serum from an individual prone to labial herpes it was usual to find that a mixture of undiluted stock virus with serum gave only a few foci, the count representing 0.1 per cent or less of those given with untreated virus. Similar methods of titrating virus or the corresponding antibody have been used for a number of viruses, but the results with herpes virus were more reproducible and satisfactory than with any other virus we have studied. Shaffer and Enders (37) also found the method convenient for the study of herpes virus-antibody reactions. In general it was possible to obtain figures for serum antibody which were accurate to within ± 50 per cent without using inconveniently large numbers of embryos. Such titrations of serum from a considerable group of unselected individuals gave results which were quite unlike those to be found with any other type of antibody test on human sera. If one measures the amount of antibody against influenza virus or against the toxins of the diphtheria bacillus or the staphylococcus, to mention three other very common types of infection, one finds some individuals—particularly children—with no antibody, others—in general those who have been recently infected—with very high amounts, and in between a continuous range of values. A frequency-distribution curve for any homogeneous group of people will, except under special epidemiological circumstances, show a peak at some intermediate value.

Our herpes results, however, had an "all or nothing" character. Either the antibody titre was very high or there was no antibody at all. This observation in itself establishes the nature of herpes virus infection as something very different from any of the other common human infections. It is evidently a continuing infection, constantly providing a renewed stimulus to antibody production. Antibodies resulting from infections of normal type—an attack of influenza say—reach a maximal concentration within two or three weeks. Then the concentration falls at rates which may vary greatly in different individuals but which in the absence of reinfection eventually bring the titres down to a low level.

In interpreting the all or nothing distribution of herpes antibody there are only two alternatives. Either there is a group of people who are and remain extremely susceptible to infection by virus in the environment—the antibody level being maintained by recurrent new infections—while another group is completely insusceptible to infection, or we have to assume that once infection has occurred the virus remains present indefinitely in the tissues, continually or intermittently providing a fresh stimulus to antibody production.

The first alternative is completely opposed to all normal experience of infectious disease; the second is in accord with all the other facts in regard to symptomatology and distribution of antibody that we have been able to collect.

Among these facts are, *first,* that in children with herpetic stomatitis there is no antibody at the time of illness but a rapid development in the subsequent weeks. This establishes the primary character of the infection.

Second. In a random group of young children those with antibody showed the same high titres as adults do.

Third. Every person tested who gave a history of being subject to herpes had serum antibody. A proportion of those who gave no such history also had herpes antibody, but most lacked it completely.

Fourth. In a few samples of post mortem serum from elderly subjects the same all or nothing findings were obtained.

Fifth. Nagler (32) has shown that a mild erythematous reaction is produced on intradermal injection of a suitable preparation of heat-killed herpes virus in children or adults subject to herpes. The reaction showed an absolute correlation with the presence of serum antibody.

The general picture which emerges is that for the average public-hospital patient in Australia, primary infection occurs at the age of one to five years in the form of a stomatitis, often with secondary lesions on skin areas heavily contaminated with saliva. The virus becomes established in the epidermal cells of the areas initially infected, and at intervals through life is recalled into local pathogenic activity. There is no evidence that infection is ever eliminated.

There are, however, variations from this general picture, and in Table 1 are some figures from Australia in regard to the influence of social status in modifying the proportion of individuals showing herpes antibody. The grouping adopted—university graduates, non-graduate laboratory assistants, and public-hospital patients—gives three groups which on the whole come from successively less comfortable homes. The contrast of 37 per cent with herpes antibody in the first group and about 93 per cent in

TABLE 1

HERPES ANTIBODY IN SERUM FROM PERSONS IN DIFFERENT SOCIAL GROUPS

GROUP	ANTIBODY PRESENT	ANTIBODY ABSENT	PERCENTAGE INFECTED
1. University graduates	10	17	37
2. Non-graduate laboratory workers	13	9	59
3. Public-hospital patients			
a. Adults	51	4	93
b. Children	56	4	93

two large groups of public-hospital patients (adults and children) is statistically highly significant.

There is a distinct suggestion from Andrewes and Carmichael's (38) study of herpes antibody in patients with encephalitis lethargica that the same influence of social level on the incidence of herpes obtains in England. In their control group of persons not suffering from encephalitis they included 8 medical students, all of whom showed no herpes antibody. The public-hospital patients who were their other subjects gave 78 per cent of positive findings.

Herpes is not a very important matter, but a study of the social and epidemiological factors responsible for primary herpetic infection could hardly fail to be intrinsically interesting and might well provide leads to the elucidation of more important problems—the epidemiology of poliomyelitis, perhaps.

There is another interesting implication of the all or nothing distribution of antibody against herpes virus. In view of the frequency of herpes lesions and the ease with which saliva must be contaminated, opportunities for infection must be widespread, particularly in winter. The fact that more than 50 per cent of people in some groups can remain free of infection till adult life must indicate that transfer of infection after infancy is relatively difficult. It is, however, by no means unknown. I have in mind one of my colleagues known to have previously had no herpes antibody, who on being retested showed a moderate antibody titre which had increased still more when tested again a month later. The explanation was simple—a few months previously she had become engaged to a man subject to occasional attacks of herpes!

The ways by which herpes infection can be transferred under natural conditions require further investigation. One can feel certain that in adults it is not transmissible by droplet infection. The simplest way to interpret the different incidence of infantile infection in different social levels is to refer it to the varying levels of hygiene in feeding. In Melbourne, at least, most cases seen in the Children's Hospital are in toddlers at an age when spoon feeding has begun. Contamination of spoon or cup by a parent's saliva and infection of minor cracks in the delicate mucous membrane of the child's mouth or lips from this source seems to be a highly probable sequence and one more prone to occur in poorer than in more comfortable homes. Other ways in which infected saliva might reach the same situation are not hard to imagine.

Probably all that is required is that active herpes virus should come into contact with sensitive (non-cornified) cells of skin or buccal mucous membrane. This is easiest in the young non-immune child, and once the infection is initiated it may extend over a considerable area of the mouth and lips and involve skin areas subject to contamination with saliva. In such children there is a definite febrile illness associated with the infection. After recovery the child is probably immune from any manifestation of herpes as severe as the initial one, but it is obvious that no absolute immunity is induced.

In older children or adults the chance of primary infection is reduced both by the lessened exposure to salivary contamination and by the possession of a thicker, more resistant, epithelium. Human subjects can be readily inoculated with herpes by scarification of the skin, even persons prone to herpes labialis and, therefore, possessing antibody. Teissier, Gastinel, and Reilly (39) were able to inoculate a volunteer with his own "spontaneous" strain of herpes virus. In such experiments they could transfer the strain from one skin area to another for 5 to 7 passages. Further passage failed, suggesting that an effective immunity had eventually developed.

There is no evidence of serological differences amongst human herpes virus strains, but there are obvious differences in their pathogenicity for rabbits. The normal method of demonstrating and isolating the virus is to place the vesicle fluid or saliva on the scarified cornea of the rabbit. Some strains regularly pass to the brain, producing a fatal encephalitis, while others produce only a local keratitis. It would be most unusual if some strains did not possess greater primary infectivity for human beings than others, and it is quite possible that, given a susceptible population under the right conditions for heavy salivary contamination plus a virus of more than normal virulence, an "epidemic" of primary herpetic stomatitis might occur. Levine, Hoerr, and Allanson (40) in 1939 described an epidemic of stomatitis involving principally young people in three summer camps in New Hampshire. They considered that the infection was probably due to herpes virus, but no laboratory studies to establish this were reported.

The conception of herpes as a specific infectious disease has been supported by all recent work on the subject (41, 42) and can be taken as fully established. The only character in which the infection is unique amongst human virus diseases is the lifelong persistence of the virus in or near the site of primary infection.

From the basic point of view of the survival of the virus we must recognize what an extraordinary biological success the herpes virus has attained. It is no mean achievement for a parasitic organism to be able to infect about 90 per cent of its possible hosts for about 90 per cent of their lives. This success is normally associated with a very trivial degree of illness in the host. Even with herpes simplex, however, the equilibrium between host and parasite is not so absolute that the virus may not break occasionally into undue activity. There are at least three well-authenticated cases of invasion of the central nervous system by the virus in the course of a primary infection: two of fatal encephalitis—in an infant (43) and in a 25-year-old soldier respectively (44); the other an instance of meningo-encephalitis with recovery in a Negro boy (45). These exceptions notwithstanding, herpes is a particularly good example of the way an infectious disease can reach a condition of equilibrium in which, despite free and effective dissemination of infection, the activities of the host are hardly discommoded in the least.

Apart from the minor differences in virulence referred to above, herpes virus strains are very uniform in character and constitute a well-defined species. There are no other human infections due to viruses at all closely related, although herpes zoster and varicella produce histologically similar lesions. Amongst animal infections there are two which are caused by viruses which have so much in common with herpes virus that they must be held to have evolved from a common ancestral form. These are the virus of pseudorabies and a herpes-like virus isolated from a fatal human infection following monkey bite and generally known as the "B" virus of Sabin and Wright (46). Sabin (47) showed that the three viruses were immunologically related, herpes and B being considerably more closely allied to each other than to pseudorabies. There is also a general correspondence in their pathogenic action in common experimental hosts, notably the rabbit and the chick embryo.

Pseudorabies has been shown by Shope (48) to be very widespread amongst pigs in the Middle West of America. The disease is highly contagious for swine, the virus being liberated from infected animals in the nasal secretion and infecting either by inhalation or in food. Symptoms are trivial and would often escape notice under farm conditions. Evidence of past infection can be obtained by testing the serum for its capacity to neutralize the virus and tested by this criterion almost all swine in the Middle West are found to have become infected before they reach the slaughterhouse.

The second monkey B virus has been little studied, but work by Hurst and myself (49) on the antibody content of normal monkey sera suggests that it is a common natural infection of both rhesus and cynomolgus monkeys. Nothing is known as to how the infection is transferred under natural conditions, but neither species of monkey shows any clinically obvious response to experimental infection with the virus. Thus all three viruses, herpes, pseudorabies, and B, are of very low virulence for their natural host.

In certain other species, however, each of the viruses can produce much more severe and fatal infections either by natural or artificial inoculation. According to Shope (50), cows may be inoculated with pseudorabies virus by being nuzzled by swine carrying the virus in their saliva. In cattle the disease produces symptoms which are vividly characterised by the farmer's term, "mad itch." Infected animals literally tear themselves to pieces, and the disease is almost invariably fatal. Similar fatal infections can be produced experimentally in monkeys and any of the common laboratory rodents. It is of particular interest that none of these highly fatal infections are contagious for animals of the same species.

The only strain of B virus which has been studied was isolated from a fatal human infection, and it is also very virulent for rabbits. Many strains of human herpes virus are also highly virulent for rabbits, producing severe local lesions on the cornea and passing regularly to the brain to give rise to fatal encephalitis. On the other hand, herpes strains have no visible effect on monkeys inoculated with them. These results exemplify the generalization first clearly stated by Theobald Smith that whenever a parasitic micro-organism has an opportunity over long periods of time to reach an equilibrium with its host species, the final form taken by the infection will be such as will not seriously interfere with the survival of the host species and virulence will be low or moderate. There is no biological reason why the virulence for *foreign* species should not lie at any level from extreme to zero. It is determined only by factors which may eventually be elucidated in physiochemical terms but which from the biological point of view are mere random structural variations of no significance for survival. It is significant, however, that when a foreign host is severely or fatally infected it is very rare to find the disease naturally transmissible to other members of the species. Ability of a pathogen to spread easily nearly always signifies that it has undergone a long period of evolution as a parasite of the host-species in question.

Herpes, then, seems from all the evidence to be an ancient disease that at least as much as any other has reached a stable configuration. It is too trivial a matter to have inspired historical research, and I know of no reference to its occurrence in the past other than Shakespeare's lines in *Romeo and Juliet.* In Mercutio's speech about Queen Mab's activities, she rides—

> O'er ladies' lips, who straight on kisses dream,
> Which oft the angry Mab with blisters plagues,
> Because their breaths with sweetmeats tainted are.

But it must be a far more ancient infection than this. Geographically, infection is almost universal; symptomatic herpes is not uncommon in natives of tropical countries with pneumonia and cerebrospinal fever, and in two sera that I tested from New Guinea natives I found antibody present in both. The only specific statement as to its absence that I have encountered is in regard to Eskimos in northeastern Greenland, where Høygaard (51) failed to see any cases of herpes in an epidemic of severe influenzal pneumonia.

V

POLIOMYELITIS

DESPITE thirty years of concentrated clinical, epidemiological, and experimental study, poliomyelitis remains as an outstanding challenge to preventive medicine. No means of preventing or curing the acute stage of the disease is yet in sight. There is equally no adequate knowledge on which to base any straightforward account of the natural history of the disease. But even with present limited knowledge it is possible to discuss some aspects of poliomyelitis from the evolutionary-ecological angle of these lectures.

Poliomyelitis, in its clinically visible form of fever and signs of central nervous system infection followed by more or less extensive paralysis, is known to be due to invasion by one of the smallest viruses. The virus can produce essentially similar infections in several species of monkey, but except for a small proportion of strains, in no lower type of animal. The more important characteristics of the virus and the pathology of experimental infections have been fully elucidated, but a great deal remains to be learnt in regard to the epidemiology of the disease.

From a biologist's point of view the most interesting developments in the study of poliomyelitis have been those concerned, on the one hand, with the predominantly intestinal location of human infection and, on the other, with the recognition of a closely similar virus disease of mice. Experimental study of the human disease can be made only in an alien host, and some of the results so obtained have led us badly astray in the past. Theiler's discovery (52, 53) of a natural disease due to a closely related virus opened up new possibilities of reaching a truer understanding of the natural human disease. The human and the rodent forms of infection have been brought still closer together by Armstrong's demonstration (54) that the Lansing strain of poliomyelitis can be transferred through cotton rats to mice, producing an infection very closely resembling Theiler's virus disease. Other poliomyelitis strains have also shown similar potentialities, notably the North African strain recently described by Schlesinger *et al.* (55). Viruses more or less closely related to Theiler's virus have been obtained from wild mice in a few instances and from the rodent known as the kangaroo rat by Melnick (56). There is a growing belief from various incomplete lines of evidence that both rodent and human poliomyelitis viruses will be found eventually to have a common evolutionary origin. It seems likely that when the relevant facts are collected we shall find that in poliomyelitis we are watching a disease in process of evolution, and that the rodent disease may provide a major clue to the understanding of that process.

To look at this suggestion more closely, we may briefly review the relevant facts about mouse poliomyelitis. The virus responsible was first isolated by Theiler from a sick stock mouse with paralytic symptoms. From the brain of this mouse a similar paralytic disease could be transferred by intracerebral inoculation to other mice. Orthodox experimental methods showed that a virus was involved which had the extremely small particle size which is found with poliomyelitis virus of human origin and which produced lesions, predominantly in the motor cells of the spinal cord, closely analogous to poliomyelitis of man or monkey.

Amongst the stock mice in which the infection was recognised, symptoms of paralytic infection were very rare, only about one in every 2000 mice being affected; but these occasional cases continued to appear, and Theiler recognised the probability that a large proportion of the mice might be carriers of the virus. The existence of such carriers was proved by Olitsky (57, 58), who showed that in Rockefeller Institute Swiss mice of about six weeks almost 100 per cent were infected without obvious symptoms. The virus was present in the intestinal contents and in the wall of the intestine. In mice under twelve days virus is rarely present, and its presence is also variable in mice over four months old; but it was very constantly present in the gut of six weeks mice. This presumably means that the infection is not transmitted *in utero* or in the milk but is contracted by the ingestion of material contaminated with faeces. The infection persists for weeks or months but is eventually eliminated, at least in a proportion of mice. Antibody response to infection has not been clearly demonstrated, and the evidence in regard to the production of specific immunity to intracerebral infection is confusing.

The epidemiological picture presented suggested that the infection is one which has reached a fully stabilized condition in mice, a condition in fact analogous to that which has been attained by herpes simplex in the human species. Symptoms are practically nonexistent, but the virus is freely liberated in a form which can infect practically 100 per cent of the new susceptibles introduced by birth. As with almost every known pathogen, there are considerable variations amongst different strains, some being much more pathogenic (by the artificial test of intracerebral inoculation) than others. Cross tests for immunological differences are probably not delicate enough to establish whether or not serologically distinct races exist.

If we turn now to what is known of how the virus of human poliomyelitis survives, we find a close parallel. In 1937 there was almost unanimity amongst workers on poliomyelitis that the virus was purely neurotropic and that it entered and left the human body at the only point where naked nerve fibres lay exposed to the environment, in the olfactory epithelium at the top of the nasal cavity. To-day no one holds that view, but it cannot be said that any similarly unanimous opinion has taken its place. Many workers believe that there are two sites of predominant importance, the tonsillar nasopharyngeal region and the small intestine. Probably a majority consider that intestinal infection is the normal means by which the virus passes from person to person, but others are disposed to think that the ease with which virus may be found in faeces is merely an indication that virus liberated in the nasopharynx and swallowed may be concentrated in the intestine.

It is clear to all that clinical paralysis is far less common than silent subclinical infection, but it is still only a matter of opinion as to what determines the occurrence of spread from the primary foci of infection to the central nervous system. Pharyngeal trauma, particularly tonsillectomy, is known to be effective, and it is possible that minor trauma—chemical, mechanical, or bacterial—in the intestine may be the determining factor in laying bare susceptible nerve tissue from which the infection can pass to the central nervous system. There are many problems here which are of the utmost clinical importance, not only in regard to what determines invasion of nervous tissue but also in regard to the intensity of the attack on the motor cells. There are many instances in clinical experience where there is clear evidence that the virus has reached the spinal cord, yet the child recovers without detectable paralysis. But this is beside our present interest. Epidemiologically, the paralytic disease whose incidence is described in vital statistics is merely a means of recognising the existence of virus in the community and of estimating to some extent its range of distribution and its relative virulence.

Much more information could be gained if we had available some convenient method by which the presence of virus could be detected irrespective of whether the clinical disease is present or not. Unfortunately, the only available method at present is to inoculate monkeys with the suspected material. No investigator can afford an unlimited number of monkeys, and there are few really satisfactory collections of data to draw on. The most illuminating study is probably that made by Francis, Krill, Toomey, and Mack (59) in investigating a tragic outbreak in Akron, Ohio. Five children of one family had their tonsils removed surgically on the same day. Twelve to fourteen days later all developed severe poliomyelitis, from which three of them died. In an attempt to identify the source of infection the history of the children's contacts with relatives and playfellows was carefully studied and faecal samples obtained from all who might be concerned. Particular attention was paid to a group of people who had very close contact with the affected children. This group contained 11 children, 7 of whom were found to be excreting virus, and 8 adults, none of whom showed virus in the faeces. The children who were found to be carriers of the virus were quite healthy, with no history of any febrile illness. The conclusion from the whole investigation was that in all probability the five victims were similar healthy carriers who would not have developed paralysis in the absence of their tonsillectomies.

When we add to the implications of such a story the fact that poliomyelitis virus can be detected with little difficulty in the raw sewage of cities like New York, Detroit, and Charleston during an epidemic season we cannot escape the conclusion that enormously larger numbers of children are infected with the virus than ever develop even trivial paralytic symptoms. This is, of course, a conclusion that had been reached many years ago on purely epidemiological considerations. The chief recent advance is the recognition of the alimentary tract as a probably primary seat of infection and as the predominant source from which the virus is liberated.

Poliomyelitis virus survives as a species in virtue of the ease with which it can become implanted in the pharynx or the intestine of children, its free liberation in the faeces, its relative resistance to destruction by environmental influences, and the readiness with which children can transfer the infection by minor faecal contamination. Whether or not the intestinal or pharyngeal infection gives rise to infection of the central nervous system is of no significance to the survival of the virus. It is probable that the major factor responsible for the epidemic appearance of paralysis is the occurrence of biologically irrelevant mutation in the virus to an unduly invasive type. As in all such problems, invasiveness is a relative term, the actual incidence of paralysis being also dependent on those still unknown factors, perhaps hormonal, which determine the susceptibility of individual children.

The general resemblance in the host–parasite relationships of the mouse–Theiler's virus system and the child–poliomyelitis virus system is almost absolute. I think we are on safe ground in assuming that the two viruses have a common origin. It is another matter to venture on a speculative attempt to interpret the relationship between the two in more detail. The only justification for doing so is that the attempt may provide suggestions for future experimental work as well as a possible interpretation of past occurrences.

The outstanding feature of the history of poliomyelitis is the way in which large epidemics have appeared only within the last fifty or sixty years (60). Sporadic cases and small outbreaks certainly occurred during the nineteenth century and probably earlier, but the modern epidemic phase of the disease seems to start with the Stockholm epidemic of 1887, when 44 cases occurred in the months from May to November. From that time onward, periodic outbreaks on an increasingly large scale have occurred in a number of countries.

There is nothing to suggest that at the present time typical poliomyelitis prevalences are in any way associated with an animal reservoir of infection, but this may not always have been the case. The relatively recent origin of the current type of the disease suggests the possibility that the poliomyelitis virus has developed new biological potentialities in the last century.

The only way to investigate such a suggestion is to provide a specific working hypothesis of the nature of such a change. The only one that offers at present is that the virus evolved primarily as a parasite of the intestine of small rodents. Faecal contamination of human food by mice and rats is only too familiar an occurrence, and if some chance mutant of the rodent virus had potentialities for human virulence, opportunity for its exercise would eventually occur. Mutability of virulence is highly characteristic of many types of virus and is well established for both human and mouse poliomyelitis viruses. Sporadic human infections might well occur from extra-human sources without being capable of easy spread to other human beings. Psittacosis is an example that springs to mind. Eventually, however, a variant may have arisen which had the power to spread freely from one human intestine or pharynx to another, and with this would come the possibility of poliomyelitis epidemics.

This suggestion for an ultimate rodent origin of the virus of poliomyelitis is at the moment pure speculation, but any speculation which immediately calls for a specific programme of experimental work is scientifically justifiable. The obvious requirement is that a systematic survey of the distribution of rodent viruses of poliomyelitic type should be undertaken. At the moment there are only two or three records of limited investigations into the natural incidence of these infections, but what has been found already suggests that such a survey may provide some fascinating and humanly important discoveries for more than one group of research workers.

But quite apart from the question of the origin of the virus, we have, even in the last fifty years, seen a very striking change in the disease which must indicate a change in the mode by which the virus survives. This is the shift in the age of maximal incidence from infancy in the early years of the century to the early school-age period at the present time. This change has been evident in North America, in Sweden, and in Australia (61). In one or two epidemics there has even been a hint that young adults may on occasion be as susceptible as children.

The age incidence of urban poliomyelitis from 1900 to about 1916 was predominantly infantile, the great majority of cases being in children under five years. This is almost exactly the same age incidence as we find for primary herpetic infection. Herpes is predominantly contracted at that age for two reasons, first because of higher susceptibility due probably to simple anatomical reasons, and second because of the relative abundance of *adult* disseminators of virus. It is often difficult to decide whether the development of resistance with age to a given infectious disease is due to normal physiological-anatomical changes or to immunological changes dependent essentially on subclinical infection. In herpes the position is relatively clear, but in poliomyelitis it is still a subject of major controversy. But there is no escaping the deduction that a high incidence of any infectious disease in infancy is always associated with an abundant source of infection in the environment. Where a virus is concerned this means that large numbers of persons (or animals) must be liberating the virus in the course of subclinical infections. In the case of poliomyelitis, for example, in the New York epidemic of 1916 the only adequate source by which we can account for the thousands of infantile infections is the adult carrier. At this period there must have been a very extensive dissemination of infection through the adult population. The relative resistance of children from the age of three upwards probably depended in part on the development of what have been called physiological barriers to the passage of virus from pharynx or bowel to the central nervous system and in part to the development of immunological resistance by earlier infection with non-paralytic strains of the virus.

The picture we are compelled to form, then, is one of widespread, probably recurring, non-clinical intestinal infection of adults and older children, reaching its maximum like other intestinal infections in the warm months. The virus had only a minimal tendency toward paralytic activity, finding only in the very young, subjects whose physiological make-up and lack of any acquired immunity made them susceptible to the clinical disease.

In looking for the changes responsible for the recent dominance of the *young school child* as the subject *par excellence* of poliomyelitis, we have to consider the possibility that the change may be related either to changes in the virus or in the human population.

A hint that the human factor is the more important is contained in the fact that poliomyelitis epidemics in recent years have particularly involved those countries with the highest standards of living and of personal hygiene—the Scandinavian countries, the United States and Canada, Australia, and New Zealand. It is in accord with all important facts to assume that the change in age incidence has been due essentially to the improvement in social technique which in the more highly civilized countries has greatly diminished the spread of intestinal infection and to a lesser degree the dissemination of pharyngeal-respiratory infections. The fairly steady decrease in the proportion of young adults showing evidence of past infection with the tubercle bacillus has been frequently mentioned in recent American writings. Our own studies on the much lower incidence of herpetic infection in persons from comfortable homes points also to the real significance of social factors in diminishing the ease of transfer of many types of infection.

There are no grounds whatever for believing that children under three are not still just as susceptible to the paralytic type of poliomyelitic virus infection as the older children who are now its chief victims. The difference from thirty years ago must be in the much-diminished frequency with which they are infected. In the investigation of Francis *et al.* (59) referred to earlier, 8 adults were found uninfected, although they came from the same households that gave 7 infected children out of 11 tested. This provides an observational backing for the deduction that nowadays adult asymptomatic infections are rare and provide greatly diminished opportunities for the infection of very young children living predominantly inside the home. Transfer of infection now seems to take place very largely from child to child. In our experience of the Melbourne epidemic of 1937–38, there was nearly always to be traced some fairly direct chain of contact between *children*. There was a very evident geographical spread of the disease from suburb to suburb, and in nearly every instance the first child to be infected in a new suburb could be shown to have visited or been visited by a child from a suburb already affected. There was no evidence of carriage of infection by adults. As with diphtheria, poliomyelitis is now spread primarily from child to child as a result of their own contacts, the actual source of virus being sometimes salivary from pharyngeal infection, and sometimes faecal.

A clear indication of the importance of social factors can be drawn from communities where the statistics for different racial or social groups are available. Hawaii provides a particularly good example (62), since the population includes four large groups of almost comparable size, Caucasians, Hawaiians, Japanese, and

Filipinos. For these groups the incidence per 100,000 and the portion of that incidence in persons over 5 years during the year 1940 were:

Caucasians	43.4 — 23.1	Hawaiians	26.04 — 4.8
Japanese	15.9 — 7.6	Filipinos	7.7 — 0.

In general the incidence is higher and the proportion of cases in older children greater in the groups of higher social status.

On this interpretation there is still the difficulty that in the earlier history of poliomyelitis 5–10-year-old children appeared to be relatively immune though they would of course be subject to at least as heavy infection as in the recent period. The two alternative explanations are, first, that the virus has become intrinsically more virulent and capable of surmounting physiological barriers that previously controlled it, or, second, that in the earlier period unduly susceptible infants were clinically infected at an early age while the others built up an immunological resistance as a result of repeated subclinical infections. The importance of deciding between these two alternatives has been recognised by most writers on the epidemiology of poliomyelitis. Apart from providing an interpretation of the past behaviour of the disease, an established solution would be of the greatest practical importance in allowing a rational approach to preventive measures.

Suppose, for instance, that it were established that natural immunization by essentially non-paralytic strains in infancy and early childhood was the reason for the relative immunity of 5–10-year children thirty years ago, and that better hygiene in the nursery today prevents that immunization and leaves the child unduly susceptible to infection by paralytic strains during its school days. There would be two alternative lines of action presented. The first would be to attempt the elimination of the virus by still greater attention to personal hygiene, the second to provide the missing immunity by the deliberate administration of non-paralytic strains to infants by mouth. On the premises we have adopted the first plan might be much more dangerous to the community than the second.

It is, of course, far too early to advocate any such action against poliomyelitis, but it is not unusual to find an ecological result of human activity which can only be remedied by a partial or complete return to natural conditions. In the case of poliomyelitis, the only tolerable solution may be that we return to the system by which a minor premium of paralytic infection in infancy must be paid to ensure immunity in older children and adults.

If, on the other hand, resistance to paralytic poliomyelitis should prove to be wholly a matter of physiological maturation, unrelated to specific immunity, it would be justifiable to make every effort to prevent infection. On this hypothesis, every year gained before infection occurred would reduce the chance of paralysis.

Since the indications for action are quite different on the two hypotheses we have mentioned, it becomes urgent that some means be developed to decide which corresponds most closely to the reality of the interaction between man and the virus of poliomyelitis.

Despite the great amount of work on poliomyelitis that has occupied the last thirty years, there are probably still unexploited means of experimental attack which might provide the information we require. On the one hand, a great deal more needs to be found out in regard to mouse poliomyelitis, both Theiler's disease and the adapted Lansing strain infection. No one has yet studied the behaviour of such strains in mouse stocks known to be free of the natural disease. If the techniques of experimental epidemiology were applied to such studies, one could hardly fail to find some potent leads toward the understanding of the epidemic behaviour of the human disease. More extensive search for similar virus infections in wild rodents might reveal some even more satisfactory "laboratory model" for poliomyelitis.

In regard to the human disease itself, my own feeling is that what is primarily needed is a long-term study of groups of infants from birth to the age of 10 or 12 years in several different types of social environment. Tests for the presence of virus in the faeces and for the appearance of virus-inactivating antibody in the blood, if adequately carried out, might in a few years replace our speculations by a body of solid fact as to the frequency and immunological effect of the subclinical infections. With the modern technique of preserving virus-containing material for years, if necessary, at — 76° C. with dry ice, such a study becomes a completely practicable affair.

This discussion of the possible evolution and development of poliomyelitis as a human disease is frankly speculative. Future experimental and epidemiological studies will probably open up new lines of approach and perhaps render some of our present dilemmas of interpretation meaningless. But, whatever increase in knowledge and understanding the future may bring, the study of poliomyelitis will still demand an ecological approach. Infantile paralysis is a changing disease that has not yet reached a standardized expression, and its manifestations may continue to change in this second half-century of its existence as an epidemic disease. Only some dynamic interpretation based on the changing requirements for survival of a virus whose environment is being altered by its host species' changing social habits can give an adequate comprehension of the phenomena. Such an ecological approach will have the additional merit of opening up new lines of fruitful investigation which we may hope will eventually allow an adequate formulation of the history and the natural history of poliomyelitis.

VI

PSITTACOSIS AND RELATED INFECTIONS

UP TO 1929, psittacosis was regarded as a very rare disease occasionally contracted from sick parrots and in all probability a result of bacterial infection by a salmonella closely related to what was then known as *B. aertrycke.* In 1929 and 1930 a sudden outbreak of cases in both Europe and North America occurred. Many of them were fatal, and attention was naturally focussed closely on both the bacteriological and the epidemiological characteristics of the disease. No great difficulty was experienced by workers familiar with the laboratory handling of viruses in isolating and characterizing the virus responsible, and particularly when methods of mouse inoculation were introduced by Krumwiede, McGrath, and Oldenbusch (63) and complement fixation methods by Bedson (64), the etiological diagnosis of psittacosis became relatively straightforward.

As has been mentioned earlier, the virus of psittacosis is of particular interest in regard to the evolution of the viruses since in many respects it is a connecting link between the smaller, more typical, viruses on the one hand and the rickettsias and bacteria on the other. It is the type of a group of viruses of generally similar character. Four of these are responsible for human disease, namely, the viruses of psittacosis, lymphogranuloma venereum, trachoma, and inclusion blennorrhoea. The others have been isolated from mice, ferrets, and cats. All are easily stained and readily observed by ordinary microscopy. The standard forms consist of spherical particles about 0.3 μ in diameter, but under suitable conditions all show larger forms and rather complex morphological changes.

Psittacosis virus is filterable through membranes impermeable to bacteria; it will multiply only in living cells, and when grown in tissue culture its main proliferation is during the period over which the host cells are actively metabolising. In all these respects it is a typical virus.

Its resemblance to bacteria can be seen, first, in its staining qualities, which are essentially the same as those of a Gram-negative bacterium or a rickettsia; second, in the character of the lesions produced, which resemble more those of a low grade bacterial infection than of a typical virus disease; and third, in a certain susceptibility to the action of bacteriostatic drugs. Psittacosis in chick embryos or mice can be modified considerably with penicillin, and although so far none of the sulphonamides are effective against it, two other viruses of the group are relatively sensitive. Both in experimental animals and clinically, infections due to the viruses of lymphogranuloma and inclusion blennorrhoea are readily controlled by sulphadiazine and to a lesser degree by other sulphonamides.

Within this group of viruses there are considerable differences in pathogenicity for laboratory animals. Trachoma and inclusion blennorrhoea seem to be specifically human diseases, the virus failing to infect any of the ordinary experimental animals or the chick embryo. The various bird viruses of the group, lymphogranuloma venereum virus, and the mouse, ferret, and cat types all produce lesions by one or other route of inoculation in mice and grow readily in the yolk sac of the chick embryo. Over the whole group, however, there is a sufficient resemblance—extending even to antigenic structure—to indicate that they form a natural group and either have a common evolutionary origin or have arisen by parallel evolutionary development.

Of the viruses susceptible to detailed experimental study, only psittacosis has been sufficiently investigated to allow a clear picture of the interaction of the virus and its hosts. Interest in psittacosis either as a human disease or as a natural infection of birds can be dated from 1929–30, when relatively large numbers of human cases were recognised in Europe and North America. The great majority of these could be traced with certainty to a few consignments of parrots from South America. There had been considerable numbers of human cases in Argentina in July, 1929, the most striking incident amongst those mentioned by Meyer (65) in his review being the infection of twelve members of a theatrical company, with two deaths, following the use of a parrot on the stage. There can be little doubt that the 1929–30 outbreaks were due to an unusually virulent strain of the virus.

Once interest in the disease had been awakened amongst the medical profession, many cases of psittacosis were recognised after 1930 which had clearly no connection with parrots from South America. The most frequent source of infection both in America and in Germany was found to be the aviary-bred budgerigar (*Melopsittacus undulatus*). This recognition led to the classical work of Meyer and Eddie (66) on the natural history of psittacosis in the Californian budgerigar aviaries. This work immediately raised the question as to whether or not psittacosis existed in the wild form of the budgerigar or in other Australian parrots. Our investigations (67) of the position in Australia showed that infection was very widespread amongst most of the common species of parrot and cockatoo, both in specimens obtained from dealers and in others specially caught for us in the "bush." Human infections included some which could be traced to such recently captured birds as well as others for which aviary-bred budgerigars were responsible. It is probable that in South America, too, psittacosis infection is widely present amongst parrots living in the natural state. The first evidence of an extensive reservoir of psittacosis outside of the order *Psittaciformes* came from the incrimination of fulmar petrels (*Fulmarus glacialis*) as the cause of a severe seasonal pneumonia in the Faroe Islands. More recently, pigeons have been shown to be widely infected in America, Europe, and Australia with virus strains differing in minor respects from those obtained from parrots. A number of proved or presumptive cases of human infection from pigeons have been reported.

Even from the limited viewpoint of the public health problem involved, psittacosis has, therefore, presented a steadily expanding field to be covered. The natural history of psittacosis and of the corresponding virus infections of birds outside the psittacine group has not been completely elucidated, but enough is known to allow a reasonable description of the conditions in parrots, at least. Two series of investigations, those made by Meyer and Eddie in California on aviary birds and by ourselves in Australia on wild native parrots, showed that the essential features were the same in both groups.

The parakeets studied by Meyer and Eddie were domesticated varieties derived from the Australian budgerigar. In the wild state, the species is found chiefly in the semi-arid areas of the interior and is noteworthy for the immense flocks in which it may be found migrating from one feeding ground to another. The wild form is predominantly green, yellow, and black in colour, but in captivity many colour variants have arisen. The bird breeds readily, and over the last fifty years large numbers have been kept as cage birds in most countries of the world. To supply the demand, there are commercial aviaries breeding the parakeets often on a large scale. In the United States the centre of this minor industry is in California, and it was here that interest in the aviary-bred budgerigar as a source of human psittacosis was first awakened.

The work of Meyer and Eddie in elucidating the conditions under which the infection spread in the Californian aviaries has been fundamental to the understanding not only of psittacosis but of the general ecological problem of host–micro-organism reactions. Their findings may be illustrated by first describing the picture seen in a typical fairly heavily infected aviary. Superficially the great majority of the adult birds appear healthy; signs of illness are chiefly evident in the young birds in the form of inactivity, loss of appetite, soiling of the vent, and nasal discharge. A variable proportion of these die. Experience will vary somewhat from year to year, but on the whole the mortality rate is inversely related to the cleanliness, lack of overcrowding, avoidance of overbreeding, and general care of the birds. Even in the best-kept aviary, however, the infection persists indefinitely and may flare into activity for no obvious cause.

The mode by which the virus of psittacosis persists in such an aviary has been determined by Meyer and Eddie as follows:—

The young birds are normally infected in the nest, probably by inhalation of infected dust; some that escape nest infection will be infected directly from other young birds when they mix outside the nest. In most birds a generalised infection results, and there are associated symptoms of varying intensity. At this stage the virus is being actively liberated in droppings and in the nasal secretion. If the young bird recovers, it gradually ceases to shed virus into the environment, but when examined by autopsy shows an enlarged spleen. Virus can usually be obtained from both spleen and kidney by appropriate inoculations. Usually by the time a bird is three months old it ceases to liberate the virus, but it has been shown that latently infected breeding females can again become shedders of virus. It is such birds which provide the source of infection for the new generation of nestlings.

The interaction between the bird and the virus is a balanced one, easily swung against the bird by unfavourable circumstances, e.g., cold weather or faulty management. There is evidence that birds from an infected aviary are more resistant to experimental inoculation with a virulent strain of psittacosis virus than are birds from clean aviaries, but acquired immunity appears to play a relatively minor role in psittacosis. Any latently infected bird seems potentially liable to lose its resistance and develop generalised psittacosis.

These Californian investigations thus uncovered a very effective mechanism by which the psittacosis virus was enabled to survive indefinitely through the aviary generations. The efficiency of the process suggests (though the suggestion may be misleading) that it is the product of a long evolutionary interaction between the two species concerned. If this is true, evidence of such interaction can best be studied in Australia, the native habitat of the budgerigar and probably the major centre of parrot evolution.

Australia is particularly rich in birds of the order *Psittaciformes*. There is a wide variety of typical parrots and parakeets, of which the budgerigar is the best known overseas, and in addition, members of two other families not found outside the Australian region are common. The lories and lorikeets are tree-living, brush-tongued parrots which subsist mainly on honey. The cockatoos are larger birds, two species of which—the sulphur-crested cockatoo and the galah—are extremely common. Most of the common species of both parrots and cockatoos are gregarious, the flocks often being very large. Budgerigars have been observed migrating in flocks that must have comprised over a million individuals. Both the common cockatoos may be found in flocks up to a few hundred strong. It is probable that this gregarious habit determines some of the manifestations of psittacosis infection in the wild.

Our observations on psittacosis in Australian parrots were inevitably guided to some extent by the relevance of particular problems to the primary aim of preventing human psittacosis. The most comprehensive set was a survey of about 200 parrots and cockatoos specially caught in the wild for the investigation. These birds represented 13 species and 3 distinct families (*Loridae, Cacatuidae,* and *Psittacidae*), and included specimens from all the five mainland states. All the common species kept as pets or cagebirds were included in the list. It is reasonable to assume that many of the birds obtained were young, but none of them were nestlings and all appeared superficially healthy. As examples of our findings, the following may be mentioned. Of 12 lories from Queensland, only 3 appeared uninfected; virus was isolated from 7, and 2 had large non-infective spleens, suggesting past infection. Two batches of galahs (rose-breasted cockatoos) were examined; one group, 27 from Victoria, showed all normal except for one enlarged non-infective spleen; the other group of 6 from Queensland all had enlarged spleens, one containing demonstrable virus. Of the two commonest groups of parrots in southeastern Australia, the rosellas (*Platycercus*) were almost free from infection, no virus being isolated from 75 and only 11 of these having enlarged spleens; grass parakeets (*Psephotus*) on the contrary were heavily infected, virus being isolated from about 40 per cent.

The figures for birds purchased from shops in Melbourne or Adelaide were essentially similar to those for newly caught birds, and under normal circumstances it seemed that most infection was contracted in the "bush" and presumably in the nestling stage.

There were a number of instances of a bird's dying of psittacosis a week or two after being captured, once with an associated human case of psittacosis. A more striking incident was the origin of several human infections from a group of white cockatoos. These had been taken in the country and sent to a dealer in Melbourne. They were kept under very insanitary conditions in a back-yard shed, and when they came to our notice, following the recognition of a human case, several had died and the remainder were all sick and heavily infected with psittacosis.

All this evidence is consistent with a life history of the virus under natural conditions wholly similar to that observed and demonstrated by Meyer in the aviary-bred birds. The normal mode of infection is evidently from the parents in the nest, and the infected young either suffer no symptoms or have overcome them by the time they are ready to fly. As in the aviary birds, one must assume that during the breeding period the females suffer a transient activation of the infection and become shedders of virus. Such a completely "invisible" cycle for the virus is too delicately balanced a process to continue indefinitely. It is inevitable that many young birds will fail to become infected in the nest, and it is equally inevitable that, either from a weakened resistance in the host or from the appearance of a more virulent mutant strain of virus, some birds will develop symptoms and die. If the infection were spread wholly by transfer from the parents in the nest it would eventually die out. With each failure of infection to occur, the number of nesting couples free of the virus would increase, and eventually the virus would be eliminated from the host species. Transfer from one adult bird to another must be assumed to occur even in the wild state, and the gregarious habit of most Australian parrots probably assures that this occurs at the roosting sites. There is very little evidence as to whether the presence of latent infection in a bird protects it against reinfection by a more virulent strain, but on general immunological principles one would be safe in assuming that it did so protect. When transfer of virus takes place between adult birds, it is those which have escaped infection in the nest that we must expect to suffer.

In 1938 there was a severe widespread epizootic of psittacosis in wild parrots of several species in the southeastern states of Victoria, South Australia, and Tasmania (68). In South Australia, deaths of parrots were abundant enough to be referred to in the daily press. One bird examined in Adelaide was shown to have died from psittacosis. In Victoria, we received information that birds were dying in the bush north of Melbourne, and an adult male king parrot (*Aprosmictus*) which had been seen to fall from a tree was sent to us for examination. It had all the signs of acute psittacosis, including a generalized skin rash. Since king parrots develop their adult plumage at about two years of age, it seems most unlikely that this bird was suffering from a recrudescence of an infection contracted when a fledgeling. A similar occurrence in the same season involved rosellas in Tasmania. Again psittacosis virus was isolated from a typical victim of the epizootic.

The virus strains that were isolated from these birds showed no peculiar characters as far as could be judged from their behaviour after mouse inoculation, and no cases of suspicious illness in human beings were reported from the affected districts. If the virus had developed excessive virulence, this heightened virulence was shown only against parrots. A point of interest from the Victorian experience was that this epizootic largely involved crimson rosellas (*Platycercus elegans*), Victorian examples of which had shown a very low infection rate three years previously. It may well have been the old story of the gradual development of a non-immune population with eventually a catastrophic spread of the virus. Those adult birds which became infected and survived would provide a fresh means by which the normal process of infection in the nest became established.

PSITTACOSIS (ORNITHOSIS) OF NON-PSITTACINE BIRDS AND SIMILAR INFECTIONS OF MAMMALS

Apart from parrots and cockatoos, the only other group of wild birds known to be infected in nature are the fulmar petrels (*Fulmarus glacialis*) of the Faroe Islands in the North Atlantic. The young of these birds are an important source of food for the Islanders, and during the months of August and September a considerable proportion of the population is engaged in the capture and preservation of the fledgeling birds. In September, 1933, an epidemic of pneumonia involving 71 persons, with 8 deaths, first called attention to the possibility of psittacosis being involved. In each subsequent year to 1937, numbers of cases ranging from 13 to 35 were recognised (69), and in 1938 the condition was definitely shown to be psittacosis by the isolation of virus both from human autopsy material and from young fulmars and by positive complement fixation tests with patients' serum (70).

The cases were very largely confined to women who were engaged in plucking the birds indoors, and there is little doubt that the virus was liberated into the air with dust and feather fragments during this process. It is certain that infection was derived from the young birds, so that there is every reason to believe that, as with the parrots, infection is transferred from apparently healthy adults to their nestlings. Haagen and Mauer isolated virus from 6 of 29 pools, each containing tissues from 8 to 10 birds; their results suggest that from 2 to 5 per cent of the young birds were infected. The variation in the number of human cases from year to year indicates that a similar variable distribution of infection could be expected amongst the petrels. As far as I am aware, no investigations of sea birds in other parts of the world have been made.

Pigeons are known to be extensively infected with a psittacosis-like virus in America, England, and Australia. Undoubted cases of the human disease have been contracted from pigeons, but the virus strains differ recognisably from parrot strains, and there is a general tendency to adopt Meyer's name, ornithosis, for the types of virus derived from non-psittacine birds. Again there is no reason to believe that the natural history of the infection differs in any essential respects from that of psittacosis.

Recent investigations have shown that a virus closely related to that of psittacosis can be isolated from cats with a respiratory type of distemper and from apparently normal mice and ferrets. The virus of lymphogranuloma venereum in man also falls into the group. Since these viruses possess generally similar pathogenic powers for experimental animals, are of indistinguishable morphology, and are serologically related, it is necessary to assume that they possess a common ancestry. As in so many other similar problems, any detailed speculation on the original hosts of the ancestral virus and on the subsequent history of its descendants must await a much wider knowledge of the distribution of the group in birds and mammals.

More distantly related to the psittacosis group are the two human viruses of trachoma and inclusion blennorrhoea. These are similar morphologically but are not transferable to the common laboratory animals or to chick embryo tissues. There is nothing on which to base any suggestions as to their evolution.

HUMAN TO HUMAN INFECTION WITH PSITTACOSIS

It is of particular interest from the point of view of the evolution of disease to note the relative frequency with which human-to-human transfer of psittacosis is being reported. Meyer (65) refers to at least 30 reported infections in nurses who had had no contact with birds, and several striking examples of chain infection are known. The most disastrous was that reported by Pinero Garcia in Buenos Aires, which involved a total of 26 cases with 12 deaths. At the hospital to which the patients were admitted, one doctor, two nurses, two other patients, and one visitor became infected. In Berlin, a chain of infection described by Hamel comprised patient–physician–intern–nurse–nurse, the first and the fourth of whom died.

These incidents are exceptional, but perhaps even more significant because of this. The high infectivity from human to human shown by the virus strains concerned would indicate that a mutant with these qualities had become dominant and retained its new characteristics throughout the incident. As might be expected from the pathology of the condition, massive elimination of virus probably only occurs at a time when the patient is seriously ill, so that normally only hospital contacts could become infected. No spreading human disease could arise under these conditions, but only a relatively minor further step might bring the potentiality of an independently spreading infectious disease. This step would be merely such as would allow liberation of effective amounts of virus from the patient at times when he was still able to mingle with the general community. A change in the character of the virus in such a direction is by no means inconceivable—inclusion blennorrhoea is a superficial mucosal infection—and given suitable conditions for its initiation a specifically human infection spreading like low-grade smallpox might arise.

There is no suggestion anywhere that this has ever occurred, but, perhaps more than any other current human disease situation, present-day psittacosis provides a direct clue to the process by which animal diseases of ancient lineage may give rise to a "new" human infectious disease.

Into all such biological transfers a large element of chance must enter. In general, an appropriate mutant must appear; this will probably not have any increased survival value in the original host and will vanish unless circumstances allow it to initiate human infection. Even then, it will only be if the human circumstances of time and place are propitious for the virus that a wide enough initial dissemination of virus will occur to give it a chance of surviving indefinitely as a human pathogen.

VII

SMALLPOX, ALASTRIM, AND VACCINIA

SMALLPOX has probably provoked more human interest than any other virus disease. In the eighteenth and early nineteenth centuries it was the most wide-spread and fatal of the diseases of childhood in Europe and North America, and the characteristic pocks on the face, with subsequent permanent scarring, made it the most easily recognisable of all the infections. Jenner's discovery of vaccination provoked enormous interest and controversy for nearly 100 years, maintaining an interest in smallpox even into the period when it was practically non-existent in Europe. It is curious, therefore, that during the modern phase of virus research, the last 20–25 years, relatively little attention has been paid to the study of smallpox as such.

Any attempt to give a comprehensive discussion of the natural history of smallpox and the related pox diseases of mammals is rendered impossible by the absence of adequate experimental and epidemiological data. This is unfortunate because one can feel that behind the fragmentary knowledge available there is an absorbing story of the evolution of a common ancestral virus into several types, each with its own host range. One can sense, too, changes in the relationship of man and the virus, both in prehistoric times, when the domestication of ox, horse, sheep, and goat was in progress, and in the historic period—from 1600 to 1800—during which smallpox reached its zenith of activity in Western countries. At the present time, we have as a basis for experiment and speculation classical smallpox in Mexico and the Far East, and the mild form, alastrim or amaas, probably endemic in Africa and appearing intermittently elsewhere. We have the vaccinia virus of the lymph laboratories, some strains of which may be descendants of Jenner's cowpox or with equal probability may be derivatives of human variola virus; and finally there are the pox diseases of domestic animals, cowpox, sheep-pox, goatpox, and horsepox.

Modern techniques of virus investigation are principally the product of English, American, and Western European laboratories, and in none of these countries is smallpox a common or important disease. Vaccination against smallpox is still an important public-health activity, and vaccinia virus is one that is particularly convenient to study in the laboratory. There is, as a result, a completely disproportionate volume of work on the properties of vaccinia virus and only very limited knowledge in regard to variola and the natural pox diseases of domestic animals.

An attempt to discuss the history of smallpox in the terms of the general point of view adopted in these lectures will therefore of necessity be highly speculative, particularly in regard to the origin of the human disease from some ancestral animal infection.

For the reasons discussed in an earlier section (p. 19), it is impossible to regard variola as a disease which has evolved wholly as a human infection; and if we are to understand its origin, relevant information must be sought amongst the related pox diseases of wild and domesticated mammals.

There are practically no reports of the occurrence of any natural disease of the pox group amongst wild mammals. There is a statement by Bleyer (71) that in Southern Brazil an outbreak of alastrim in the natives was associated with a severe smallpox-like disease of monkeys (*Cebus* and *Mycetes*). Councilman (72) refers to a similar report of smallpox in man and monkeys near Panama in 1841. Findlay (73) mentions an old record of a sheep-pox outbreak in France in the eighteenth century said to have been associated with a severe pox in wild rabbits which exterminated whole warrens. Epidemics of rabbitpox such as those described by Greene (74) in laboratory rabbits in New York may represent the activation of a normally latent virus specific for the species, but there is always the alternative that the virus was derived from a strain of vaccinia.

All the well-established animal pox diseases are of domestic animals and (with the exception of rabbitpox) of the domestic ungulates—cow, horse, sheep, pig, and camel.

If smallpox is not a primarily human disease, it is natural to look for its original host in one of the wild ungulates from which domestic stock are derived. Without some positive evidence of an enzootic disease of this type in some extant wild animal of the order, such a thesis can only be a slender speculation. There is sufficient evidence that cowpox (of Jennerian type) and horsepox are sufficiently closely related to variola–vaccinia to make it reasonably certain that the three viruses must have had a common ancestor. There is, however, at least as high a probability that the horse and cow infections are derived from the human disease as vice versa. The spread of cowpox amongst dairy cattle seems to be generally ascribed to transfer of the virus from cow to cow by the hands of the milker.

Sheep-pox is distinctly further removed from human variola and vaccinia and gives every indication of having been a well-established specific infection of sheep for many centuries. As is usual in such diseases, the acute widespread fatal epizootics probably represent essentially the introduction of virus into a non-immune population. The reports from French literature (75) that Algerian sheep showed very low-grade infections but would almost regularly transfer virulent infection to French sheep would indicate that in Algeria the disease was truly endemic and had been established there for long periods. Despite some contradiction in the literature, it seems certain that sheep-pox cannot be converted into vaccinia and is not infectious for man. Goatpox can also give rise to epizootics and has a general resemblance to sheep-pox, although the two types are both relatively host specific.

The mammalian pox diseases seem to offer at the present time one of the most inviting fields for the study of variation and host specificity in a group of related viruses. There are two technical aspects of such a study that we have explored to some extent in Melbourne—growth of pox viruses on the chorio-allantois of the chick embryo and agglutination of red cells by vaccinia virus. Neither of these methods has been utilized to the full in comparative studies. It is known that variola, vaccinia, cowpox, and sheep-pox will grow and produce lesions on the chorio-allantois, and it is likely that all mammalian pox viruses could be adapted to grow adequately in this situation. With properly adapted strains and appropriate immune sera, cross-neutralization titrations could be carried out by the chorio-allantoic method. This would provide quantitative data in regard to antigenic relationship which is at present wholly lacking. Serological study might be still further simplified by an extension of Nagler's work on haemagglutination by vaccinia virus to other members of the group (76). Nagler found that vaccinia virus from chorio-allantoic membrane lesions agglutinated erythrocytes from approximately 50 per cent of adult fowls but was inactive against all other types of red cells as well as against embryonic chicken cells. Calf lymph virus failed to agglutinate cells, apparently because of the presence of an inhibitory agent. The method was applied to the estimation of circulating antibody in a large group of vaccinated children and adults as well as to a few rabbit and calf immune sera. This is a simple *in vitro* method of serological study which might well be applicable to the other pox viruses that can be grown on the chorio-allantois.

North (77) has recently published an account of limited experiments along these lines with variola virus. This strain agglutinated fowl red cells, although not so actively as vaccinia virus, and the agglutination could be inhibited with specific immune serum.

When such methods have been improved and applied to a wider range of pox viruses, it should become possible to make much more effective surveys of the incidence of diseases of the group in wild animals.

Well-known examples of the application of serological surveys of wild animals to elucidate the epidemiology of certain virus diseases are to be found in reference to yellow fever and the mosquito-borne encephalitides. Two examples from my own experience, not so directly concerned with human disease, were the recognition in one batch of wild rabbits of an exceptional number with high titre staphylococcal antitoxin and the finding with Weston Hurst that a relatively high proportion of *Macacus rhesus* show antibodies against herpes and B viruses (49). There was no opportunity to study the significance of the staphylococcal antitoxin, but a discussion of the herpes antibody in monkeys has been given in Chapter IV. There are two groups of animals from which an origin of the pox viruses might reasonably be sought. In view of the existence of pox diseases of each of the domesticated species of ungulates, it would be natural first to look for an ancestral form amongst wild ungulates related to those from which the domestic breeds have been derived. No extant disease of the type seems to have been described for such animals, but this is far from excluding the possibility of its existence. If an infection were present like that described for Algerian sheep by Waldteufel and Nocard (75), with very mild lesions and without definite epizootic manifestations, it is most improbable that its existence would be recognised by casual observers.

The other possibility is that variola may stem from an enzootic disease of African monkeys. Variola virus direct from human material will infect a number of types of Asiatic and African monkeys and no other type of laboratory animal. Brinckerhoff and Tyzzer (78) produced experimental infections of both cynomolgus monkeys and orang-utans, but these infections were not naturally contagious for other animals of the same species. In South and Central America, there have been several circumstantial reports of a severe pox-like disease in monkeys being associated with epidemic smallpox or alastrim in human beings, but no laboratory confirmation of the nature of the disease. These indications that monkeys are more susceptible to variola than any other type of mammal would be compatible with a primate origin of the disease, but there is no evidence to suggest that any enzootic disease of the type exists amongst monkeys at the present time.

In looking for evidence of inconspicuous pox diseases in these or other groups of wild animals, serological methods would normally be the only practical ones. In such work, Nagler's technique, using vaccinia and any other pox viruses which may be found capable of giving specific haemagglutination reactions, would probably be of the greatest value.

Only when comparative studies of this sort have been made will it be possible to give a reasonable account of the probable evolution of the pox viruses.

In the absence of the necessary experimental data, it is impossible to give even a reasonable guess as to the type of mammal from which the virus smallpox was originally derived. Nor is it possible to say at what period of human history, or prehistory, smallpox developed its power of epidemic spread. The early history of smallpox epidemics (before 1500) is also extremely vague, and it is usually impossible even to be certain whether epidemics like the plague of Galen in 165–168 A.D., later ascribed to smallpox, were really examples of the disease or not. It is quite impossible to use the accounts of such epidemics as a basis for discussing possible changes in the character of the disease. There are statements that smallpox is of very great antiquity in China, but most writers refer to the outbreak of smallpox in an Ethiopian army at the siege of Mecca in 569 A.D. as the first indubitable epidemic recorded. The classical Arabic account of smallpox by Rhazes was written in the early eleventh century and the first clear descriptions of the disease to appear in European medical writings were undoubtedly derived from Arabic sources. This, taken along with other evidence, suggests that during the Middle Ages smallpox was rare or non-existent in Europe. Whatever may have been the experience in the classical period, there seems to be every reason to believe that the modern history of smallpox in Europe began with its introduction by the Saracens. According to Creighton, there is no valid evidence that either smallpox or measles existed in England in the fourteenth and fifteenth centuries.

A brief discussion of the history of smallpox in England and Scotland since the sixteenth century may provide a picture of the changing character of the disease, with perhaps some indication of the changes that may have been taking place concomitantly in the characters of the virus. The facts on which this discussion is based are drawn wholly from Creighton's *History of Epidemics in Britain* (79).

The first English record is in 1514, and at this time the names measles and smallpox (variously spelt) were used together or indiscriminately. Kellwaye in 1592 described smallpox in terms which suggest that the disease had become fairly common and that its normal form was indistinguishable from present-day alastrim. He differentiates it fairly clearly from measles. Other evidence from the sixteenth century indicates that smallpox was to be seen both in adults and in children and that it was popularly regarded as of no great infectiousness or consequence. It was still the small pox in contrast to the great pox (syphilis).

In the seventeenth century, evidence of increasing virulence begins to appear, with references to the likelihood of facial disfigurement in young women attacked by the smallpox, and with sharp increase in the number of accounts of smallpox in members of the nobility. The first notable epidemic in London was that of 1634, when 1354 deaths were ascribed to smallpox in the bills of mortality. During the Commonwealth, the London figures averaged nearly 1000 deaths per annum, and there was much smallpox of a more severe type elsewhere. After the Restoration, smallpox became still more extensive and fatal, its victims including a brother and sister of Charles II within a year of his return. During this period it was noted by Willis that smallpox was less severe in children than in adults, "the sooner that anyone hath this desase the more secure they are, wherefore children most often escape [death]."

During the eighteenth century, smallpox was solidly endemic in the great cities, London recording around 2500 deaths per annum, with relatively small variations from 1720 to 1800. The incidence of the disease was almost wholly in childhood. In Manchester around 1770, for instance, 559 of 589 deaths from smallpox were in children of 5 years or less. In Glasgow, at the end of the eighteenth century, about one-third of all deaths under 5 were from smallpox.

At the beginning of the nineteenth century, smallpox began to lose its position as the most important cause of death in early childhood, and an increasing incidence of smallpox deaths appeared in the young adult age group. This change appeared earlier in the country centres than in London, and it was well established in the great Continental cities before it was evident in London and Glasgow. It is characteristic of recent smallpox, e.g., the Sydney epidemic of 1913, that even in unvaccinated populations the incidence tends to be greatest in the young adult group.

The general picture of the rise and fall of smallpox over four centuries in England shows, first, a mild disease spreading more readily amongst adults than in children, then its gradual development in the great cities to an endemic disease with an almost universal incidence in children under 5 years, and finally a reversion to its earlier type.

Some part of these changes must be ascribed to changes in the social circumstances, reaching a peak in the early years of the Industrial Revolution; and the development of vaccination almost certainly played its part in the decline during the nineteenth century. But in addition one must postulate some changes in the intrinsic virulence of the virus strains dominant at different periods. In the endemic period, the virus was of high virulence and had some characteristic that favoured its ready spread amongst children; the escape of older children and adults was due solely to their previous immunization by experience of the disease. The milder smallpox of the sixteenth century, with its predominance of attack on adults, probably owed this characteristic simply to a generally less efficient means of transfer. This allowed a large proportion of the population to escape an immunizing infection in childhood. When more virulent mutants arose, they found conditions in the cities suitable for their continued survival in virulent form. The subsequent change to a type of disease again showing a predominantly adult incidence is more difficult to account for. It is hard to see why within a decade or two, smallpox was replaced by measles as the major cause of death in childhood, particularly as the evidence indicates that there was no real decrease in case fatality until late in the 1880's. There may have been factors now quite unrecognisable at work, but it seems likely that the economic prosperity during the Napoleonic wars and the introduction of vaccination together made it more difficult for the virus to spread from person to person. Big epidemics remained possible until 1870–71, but the changed host situation rendered it impossible for smallpox to maintain its endemic character. It was virtually eliminated from England in the twentieth century until its re-entry as alastrim in the 1920's.

Any discussion of the present position must necessarily be concerned largely with recent epidemics of this mild smallpox or alastrim and with the relationship of this disease to classical variola. Mild smallpox, lacking the gross pustulation of the secondary stage and with a very low mortality, has been observed at various times: Jenner described such an epidemic in Gloucestershire in 1789. In 1865 there was a widespread epidemic involving Negroes in Jamaica, and in 1903 the disease was described under the name amaas or Kaffir milkpox amongst South African natives. Alastrim epidemics have been particularly associated with Negro populations, but there seems to be no reason to regard the Negro as intrinsically more or less susceptible to smallpox than the European. There is, however, no doubt that smallpox has been widely prevalent in Africa since time immemorial. Ethiopia was regarded as the traditional home of the disease, and from the data available it seems likely that amaas has been endemic in the Negro population of South Africa for many years—possibly for centuries. The Australian epidemic of 1913 had the typical mild character of an alastrim epidemic, and there was another extensive outbreak in Jamaica in 1920–21. Large numbers of cases occurred in England in the period 1921–25.

The relationship between alastrim and classical smallpox requires more adequate experimental study. There is no serious doubt that both variola and alastrim viruses can be transmitted to monkeys, producing essentially similar lesions, and that Jennerian vaccination in man is an adequate protection against either. The major clinical difference is in regard to the secondary toxic stage, associated in classical smallpox with streptococcal infection of the mucosal lesions and to a less extent of the skin pustules. This stage is practically absent in alastrim, and as Findlay (73) suggests, the real difference may be related more to the associated streptococci than to the virus. It would be of very great interest to have the results of a careful bacteriological study of a classical smallpox epidemic. Should it be found that haemolytic streptococci are regularly present in the throat lesions, and of a single Griffith type, it might be possible to deduce that classical smallpox represents the activity of virus plus a specifically associated haemolytic streptococcus, while alastrim is due to the virus alone. The history of scarlet fever indicates that at times very highly virulent streptococci with specific localizing capacity may appear, and it is not unreasonable to suppose that this may also occur in association with smallpox virus.

The alternative, that the difference lies essentially in the virus, is a more orthodox view. The virulent strain would predispose to the secondary septic invasion of the lesions by any available streptococcus or staphylococcus, perhaps by causing such a degree of cell autolysis as would allow free growth of the cocci. In alastrim, the primary skin lesions are less destructive and have no more tendency to become infected with cocci than have those of varicella. On such a view, the infecting cocci in classical smallpox cases would be expected to be heterogeneous in type and to correspond more or less closely with the predominant throat strains in the community concerned.

In the absence of direct evidence on the point, it is difficult to make any deductions from epidemiological data. It is very rare for an alastrim epidemic to give rise to a group of virulent smallpox infections. One such occurrence is on record in regard to a small outbreak in England (Davies, quoted by Findlay, 73), and there is a suggestion of its occurrence in the West Indies in

1920–21. The initial epidemic of alastrim in Jamaica was very mild, the death rate being less than 0.5 per cent; but after the importation of the disease into Haiti in August, 1920, there was a steady rise in mortality, so that the total mortality of patients in hospital reached 9 per cent (80). Whether the increased mortality was due to unfavourable nutritional or social conditions, to association with more virulent streptococcal concomitants, or to a real change in the nature of the virus must remain an open question. One would guess that the third alternative is the least likely.

Probably the simplest interpretation of the variola–alastrim relationship is that minor variations of smallpox virus are occurring constantly. Individual epidemics are probably initiated in the last analysis by a very small infecting dose, possibly a single virus particle, so that it may happen that a virus strain with no special qualifications for eventual survival will find an opportunity to produce an epidemic. Each epidemic will have more or less uniform characteristics except insofar as it finds differences in population resistance or in associated pathogens. In the modern period those strains will have particular survival value which by their mildness do not provoke public alarm and expeditious public health action in quarantine and vaccination. Conversely, any appearance of the virulent strain will provoke effective action for its extermination. It does not seem necessary to seek any more subtle explanation for the predominance of alastrim in recent years.

THE RELATIONSHIP OF VACCINIA TO VARIOLA

Ever since Jenner's first announcement of his method of vaccination, the relationship of vaccinia virus to the virus of true smallpox has been a matter of controversy. Ledingham (81) has discussed the question of the origin of the standard strains of vaccinia virus used in the calf lymph establishments and has pointed out the virtual impossibility of knowing whether these are derived from Jenner's cowpox strain or spring from smallpox virus picked up in the course of arm-to-arm transfer in the early nineteenth century. When we confine ourselves to modern work, it appears to be adequately proven that some strains of variola at least can be "converted" into vaccinia by consecutive passage through monkeys to rabbits and calves. No thorough study seems to have been made of the processes by which the change takes place, and since the phenomenon is the classical example of virus variation this absence of knowledge is to be regretted.

There are a number of apparent analogies between the variola–vaccinia relationship and the O–D change in influenza virus which is described in a subsequent section (p. 110). In both instances, a highly infectious human pathogen, with very limited power of transfer to experimental host species, is transformed into a relatively stable type, less virulent for human beings but with a wider range of susceptible hosts. If this analogy is real, the change from variola to vaccinia virus must also represent a discontinuous mutation—possibly made in one step, possibly requiring a series of intermediate steps. From the available descriptions of the process, it seems likely that the essential change is made in a single step that occurs with a very small frequency. Transfer of a strain of variola virus through many generations on the chorio-allantois did not in Nelson's hands (82) result in the appearance of vaccinia. Similarly, Horgan (83) found that with continued passage in the monkey, variola virus retained its characteristics practically unchanged. If these findings are generally applicable to all strains, the inference is that neither in the chick embryo nor in the monkey does vaccinia possess any significant survival advantage over variola. Only in the rabbit skin will any mutants to vaccinia find an opportunity for selective overgrowth which will rapidly result in the complete replacement of the original virus.

Vaccinia virus itself can give rise to secondary variants by appropriate transfer in experimental animals. Amongst the variants which have been described are strains capable of killing mice regularly on intracerebral injection, Levaditi's neurovaccine with a heightened virulence for the central nervous system of rabbits, Ledingham's intradermal passage strain, and Armstrong's heat-resistant variety.

In recent years, much careful experimental work has been carried out with vaccinia virus; only influenza A virus (in its adapted laboratory form) has been subjected to anything approaching such a detailed study. The physical and chemical characteristics of vaccinia virus are known with considerable accuracy, and its immunological behaviour in experimental animals has been exhaustively analysed (3). Most of the information obtained, however, seems at the present time curiously irrelevant to human problems or to any understanding of the biological relationship of vaccinia to the natural pox diseases. With future comparative studies of other viruses, it may become possible to utilize the information available about vaccinia virus, but at present all that it can add to the biological picture is the assurance that, physiochemically, vaccinia virus is a simplified but still reasonably complex living system that might well represent an intermediate stage in an evolutionary degeneration from bacteria to the smaller viruses.

From our present point of view, the biological significance of vaccinia is still almost the same as it was once Jenner's work had been established. Vaccination is an effective method of creating a community resistance to the spread of smallpox so that variola virus entering the population is unable to establish itself and is rapidly exterminated. Even before vaccination was introduced, or in its present-day absence, variola is not an explosively spreading disease like influenza, and it also has difficulty in establishing itself as an endemic disease. Only in the eighteenth century, and even then only in the cities, was smallpox truly endemic in England with the characteristic feature of an endemic human disease, a predominant attack on childhood. Any change in the epidemiological circumstances which rendered spread more difficult might well have a cumulative effect in breaking down the endemic state. It is at least a reasonable working hypothesis that vaccination was the most important of the factors concerned in the virtual disappearance of classical smallpox from European civilizations during the nineteenth century. It is probably equally likely, as has been indicated earlier, that administrative and psychological vagaries in the use of vaccination have been responsible for its replacement by alastrim.

VIII

YELLOW FEVER

Amongst human infectious diseases only plague can rival yellow fever in its influence on human history and in the sinister impression it has made on the human mind. For three centuries it was persistently associated with the slave trade between West Africa and the Caribbean region and it played probably a greater role than any other factor in determining the course of war and piracy in the West Indies during the eighteenth and nineteenth centuries. Yellow fever was notoriously a disease of tropical ports and of the ships, particularly slave ships, that visited them. The two famous literary legends of the Flying Dutchman and the Ancient Mariner both reflect stories of ships stricken with the disease (84).

The first great step toward the understanding and control of yellow fever was made by the famous experiments of the United States Army Commission in Cuba in 1900–01 which showed that the responsible agent was transmitted by the mosquito now called *Aëdes aegypti.* The essential conditions for the transfer of infection were rapidly elucidated, and long before the nature of the responsible organism was discovered appropriate public health action had cleared Havana of the disease and made possible the construction of the Panama Canal. In 1928, Stokes, Bauer, and Hudson (85) initiated the modern phase of yellow fever study by showing that the disease could be experimentally transmitted to rhesus monkeys.

By 1930 a great deal had been learnt about the virus, and it seemed that all the essential knowledge had been gained to ensure the rapid extinction of yellow fever from the globe. The disease was known to be due to infection by a virus which multiplied particularly in the cells of the liver and for a brief period at the height of the disease gave rise to an enormous concentration of virus in the blood, as many as 10^{10} infectious doses per cubic centimeter (86). The virus was spread by the mosquito *Aëdes aegypti* which required to feed on a patient during the first 3 days of fever if it was to become infective. The mosquito could not transmit infection by its bite until a period of about 12 days had elapsed after the infective feed. *Aëdes* is essentially a domestic mosquito, the adults being found around human habitations and the larvae developing principally in small collections of rain water. These characteristics made it one of the easiest mosquitoes to eradicate from populous areas. On the basis of these facts, the main centres of yellow fever around the Caribbean Sea had been eliminated, and the removal of South American and West African foci seemed to require only an adequate amount of time and energy.

At this period, however, the methods needed for the closer investigation of the natural history of yellow fever were being perfected. In 1930, Theiler (87) had developed his neurotropic strain of the virus in mice and made it a practical procedure to test large numbers of human sera for the presence of antibody (88, 89). This made it possible to detect with certainty areas in which infection amongst native populations was occurring even though the typical clinical manifestations were rare or absent. About this time, too, the viscerotome technique for obtaining samples of liver for histological study from persons dead of suspicious fever was introduced by the Rockefeller Foundation workers in Brazil (90). By these methods it became possible to make much more effective epidemiological surveys of the tropical regions of Africa and the Western Hemisphere and to determine accurately the present and immediate past presence of human infections due to the virus. With the accumulation of such data the illusion of an easy conquest of yellow fever vanished precipitately.

Persons whose blood gave indubitable evidence of past infection with the virus were found to be distributed over vast areas in South America and Central Africa, including areas in which no clinical yellow fever had been reported (91) and others in which *Aëdes* mosquitoes were known not to be present (92). The story of yellow fever has, therefore, had to be drastically rewritten, and in the process it has become much more in harmony with our current ideas of the origins of virus disease in man.

The position at the present time seems to be that in various parts of tropical Africa and South America the yellow fever virus is transferred by jungle-haunting mosquitoes (not *Aëdes aegypti*) to and from a number of species of monkey, with a possibility still open that other types of mammals or birds may also constitute part of the reservoir of infection. Men working in jungle clearings are frequently infected in South America, and many of the cases are fatal (93). These are the cases in which the diagnosis is made primarily from viscerotome material. As in so many other instances, infection of human beings under these circumstances is a mere biological accident. It is too early to be dogmatic about the essential natural history of the virus in the jungle. Monkeys have been found dead of the infection in the wild (93, 94), a circumstance which at first sight would suggest that they are also only an accidental host, but the occurrence may have been only one of the destructive phases which occur sooner or later in any host–parasite relationship. The standard relationship might well be one in which illness in the monkey host is trivial. A great deal more work will be needed on the obviously complex ecological relationships of the mosquito vectors and large number of potential mammalian hosts in tropical rain-forest areas before a satisfactory picture of the condition is available.

But even on the current evidence it has become extremely probable that the classical yellow fever of the slave-trade ports is a relatively recent development of a disease which has evolved primarily in the African or American jungles. An example of the way in which jungle yellow fever can initiate an epidemic of classical urban type is to be found in the description by Walcott and others (95) of an outbreak in the town of Cambara (Paraná, Brazil) in 1936. A labourer working in primitive jungle some miles from the town suffered a clinically typical illness subsequently confirmed serologically. He lived and was cared for during his illness in one of a small group of houses in an *Aëdes*-infested area of the town. A second case appeared in this area 17 days later, and in the next month 25 of the 47 inhabitants of this area were attacked. Anti-mosquito measures were instituted, and no spread beyond the area to the rest of the town occurred and none of 108 samples of blood from persons outside the area gave any evidence of infection.

If such an occurrence took place in 1936, there seems no reason at all why it could not have taken place equally well in the sixteenth or seventeenth century. In fact, if we study the history of yellow fever as recounted by Scott (84), the existence of jungle yellow fever seems to provide the essential key to its interpretation. The retrospective diagnosis of acutely fatal fevers in the tropics or elsewhere is notoriously difficult, even when the historian seems to provide a wealth of clinical data. The plague of Athens described in detail by Thucydides is a classical instance. It might have been an abnormally virulent type of any one of half a dozen diseases; some have even suspected yellow fever. Even in our own day, Noguchi's work on yellow fever was completely misleading because of the inability of his clinical colleagues to differentiate between leptospiral jaundice and yellow fever. So, as Scott points out, the diagnosis of yellow fever from historical material must be more a matter of judgment than of certainty. The most useful distinguishing points are the occurrence of the disease in regions known to be favourable for *Aëdes*, the high mortality in European adults with much less effect on children, and the relative insusceptibility of Negroes.

It is generally accepted that the first undoubted descriptions of yellow fever in the Western Hemisphere date from the epidemics in Yucatan, Barbados, and Cuba in 1648–49. The symptoms seem to have been typical, and there is the illuminating remark that in Yucatan "God spared the young and innocent but not the sinful elders." On the other side of the Atlantic, yellow fever is not definitely recognisable until 1778, the epidemic of that year in Senegal being clearly described by Schorre. The disease has certainly been present on the west coast of Africa ever since, and there is every reason to believe that it had been present for centuries previously. The unhealthiness of the coast had been notorious since the first visits of Europeans, and it is a reasonable surmise that the loss of almost a quarter of his men by Drake as early as 1585 was due to yellow fever (96). The resistance of the west coast Negro to the disease also speaks for the existence of yellow fever in tropical Africa from the earliest times. In part this resistance probably depended on the acquisition of immunity after mild infection during childhood; in part, on a real racial resistance.

If our general thesis is correct that none of the acute virus diseases can have evolved primarily as diseases of the human species, it is obvious that we have to look for the origin of yellow fever as a mosquito-borne disease of jungle mammals. On the historical and current epidemiological evidence, Africa seems a far more likely place of origin than America. An attempt to reconstruct the story of yellow fever will still require a certain amount of speculative mortar to hold the facts together, but the general shape can hardly be in doubt.

The first stages of the evolution of the yellow fever virus are beyond conjecture. Perhaps the fact that the virus has a cyclical development in the arthropod vector (97) might prejudice us in favour of its evolution from a protozoan ancestor rather than from a bacterium. As far as I am aware, no studies have been made of the histology of yellow fever infection in *Aëdes*. Perhaps a hint might be obtained from such studies in regard to the evolutionary origin of the virus. As it is, we must start with the disease in more or less its current form, the virus surviving in some of the African jungle mosquitoes, perhaps species of the genus *Aëdes*, and in the blood and tissues of wild animals, in all probability monkeys. The ecological association was and is a loose inconstant one, owing to the large number of possible host and vector species. A great many species of mosquito can be infected with yellow fever virus in the laboratory, and many species of mammal are potentially susceptible at least to subclinical attacks. This should allow the appearance in nature of a variety of mutant strains of virus selected out of the range of possible variants by the special characteristics of one or other combination of insect vector and vertebrate host. It is not surprising, therefore, to find evidence for the existence of virus strains of extremely variable degrees of pathogenicity for man, or of differences in the readiness with which the classical vector of seaboard cities, *Aëdes aegypti,* becomes infected. In the Sudan, for instance, serological tests made in 1935–36 showed regions in which up to 79 per cent of the inhabitants had been infected with yellow fever, although there was a complete absence of any history of the clinical disease (91). In much the same districts, on the other hand, there were severe outbreaks of classical yellow fever in 1940–41 (98). Again, in Brazil there have been outbreaks of influenza (i.e., of a benign undifferentiated short fever) which appropriate laboratory tests indicated were really epidemics of yellow fever of abnormally low virulence (99).

In the jungle, man is only an incidental host for the virus, but when an opportunity arises the virus can become involved in a totally different ecological association, the tropical seaport with its hosts of *Aëdes aegypti* breeding in domestic water containers and its crowded human populations. In such a set-up the virus becomes a true human parasite, repeated passage through man being necessary for its survival. This was the state of things which developed in the slave-trade ports in West Africa and America during the seventeenth and eighteenth centuries. But it must be recognised that there are important limitations on the effectiveness of this new ecological association as a means for the permanent survival of the virus species. The human patient can serve as a source of virus only for a period of 3 or 4 days. Thereafter, irrespective of whether he dies or recovers, he can play no part in the natural history of the virus. Immunity, with perhaps rare exceptions, lasts for life. In any community changing only by the normal processes of birth and death, the infection will inevitably die out within a year or two. Only when there is a more or less continuous influx of susceptibles can the disease remain endemic. The history of the West Indies epidemics bears out this contention admirably. Cuba suffered its first serious epidemic and apparently its first contact with yellow fever in 1649, and the disease remained prevalent till 1655. Thereafter it was absent until a fresh outbreak in 1761 which caused a very high mortality amongst the troops of an English expedition against the island. From this time on yellow fever remained endemic in Havana until it was stamped out by Gorgas in 1904–05—a persistence probably to be related to the steadily increasing travel of the period, with a continuing supply of new susceptibles. Infection was repeatedly introduced into Spanish ports, and large local epidemics resulted, as at Cadiz in 1730, but the disease never became endemic.

So, from the historical record, one pictures the virus surviving indefinitely in the African jungle and breaking out whenever conditions of *Aëdes* prevalence and a sufficient density of susceptible human population allowed it to produce port and ship epidemics of yellow fever. Infected *Aëdes* were constantly being transported to America in the slave ships. It is even possible that the species *Aëdes aegypti* was imported in this way into the Western Hemisphere (100). Yellow fever became implanted around the Caribbean, flaring up irregularly as human activities—commerce, slaving, war, or piracy—provided sources of infection and susceptible populations. It is impossible to say when the yellow fever of *Aëdes* and the ports found an opportunity to revert to its jungle habit in the American forests. There is in fact nothing to *prove* that the virus was not always present in America, but it seems extremely unlikely. There is reasonable certainty that a 1907 outbreak in Muzo, Colombia, was due to infection by mosquitoes other than *Aëdes,* but clear recognition of jungle yellow fever dates from 1932 (92). In 1935–36 there was an extensive prevalence of the disease over a large area of eastern and southern Brazil in regions never known previously to have suffered. This naturally suggests that the jungle yellow fever has been introduced into the Amazon forests relatively recently and has not yet found its final distribution. Deaths presumably from yellow fever amongst wild monkeys, noted first in Venezuela in 1914 and recently in Brazil, also point to an unstable developing ecological association.

No adequate reason seems to have been given for the absence of yellow fever from the East African seaboard and from the tropical regions of Asia and the East Indies. *Aëdes aegypti* is abundant, and strains from these areas have been shown to transmit yellow fever experimentally (101) and to show no obvious physiological differences (102) from West African or American strains. Dengue can be spread by this mosquito throughout its range. Dudley (103) suggested that the *Aëdes* of the Indian Ocean region is a physiological subspecies incapable of transmitting the virus in nature, but the experimental evidence seems on the whole opposed to such a view. This problem of the past and present freedom of the East from yellow fever is of outstanding theoretical and practical importance. Until it has been solved, the possibility remains both of extensive *Aëdes*-borne epidemics in Asia and of the establishment of a permanent jungle reservoir in that region.

IX

INFLUENZA

IT IS THE THEME of these lectures that the understanding of infectious disease requires that it be studied essentially as an ecological problem of interaction between species. Sometimes, as with herpes simplex, the interaction can be described in relatively simple fashion; sometimes, as in poliomyelitis, an obviously complex situation remains obscure mainly from technical difficulties which hinder detailed study. With influenza we have a still more complex problem. Recent work has made influenza virus the most amenable of all viruses to detailed laboratory study, but as yet only a very incomplete picture of the natural history of influenza has emerged.

Modern research has succeeded in sorting out from the mass of epidemic febrile infections two etiologically defined diseases, influenza A and B. Influenza A in its typical epidemic manifestations is fairly well understood; influenza B is considerably more obscure; while in regard to the large group of influenza-like infections which cannot be ascribed to influenza viruses A and B, we are almost completely in the dark. Discussion of influenza viruses must necessarily be limited to the two known types, but it is unlikely that we shall ever understand influenza properly until the numerous clinically similar respiratory infections not due to influenza A or B can be sorted out and studied.

It is unnecessary to detail the methods by which influenza virus can be isolated and characterised, but it is worth while stressing the ease with which the virus can be handled in the laboratory. Once certain preliminary difficulties are overcome, the virus will multiply readily in the lungs of mice and in the allantoic cavity of chick embryos. Infected allantoic fluid is a particularly rich and convenient source of virus. Titration of the virus in such fluids has been made extremely simple by Hirst's discovery of the power of influenza virus to agglutinate chicken red cells. In place of expensive animal inoculations, it is now possible to use a simple test tube method for the titration either of virus or of its specific antibody. With the development of these methods, the study of virus influenza in the laboratory becomes as simple and practicable a matter as a similar investigation of diphtheria or typhoid fever.

Influenza viruses A and B are almost strictly pneumotropic; in other words, they will invade and multiply in the cells lining the upper and lower respiratory passages and—with the unimportant exception of the conjunctival epithelium—in no other type of accessible human cells. This fact seems to be basic to any understanding of the character of influenza, either in its clinical or in its epidemiological manifestations. The accepted interpretation of a typical acute attack of influenza is that the virus establishes a focus of infection somewhere in the respiratory tract. Here an epithelial cell is invaded and the virus multiplies, causing necrosis and liberation of virus on to the epithelial surface. Further cells are infected and the process continues, sweeping over the epithelial surface like a prairie fire. This is almost certainly an unduly simplified picture; there are regions of the respiratory epithelium much more sensitive to infection than others, notably the bronchiolar epithelium. Such qualifications, however, detract nothing from the essential conception of influenza as a *surface* infection. The *speed* of spread over the epithelium is probably the main factor which determines the acuteness and severity of the initial symptoms. The typical "toxic" onset is to be ascribed in all probability to the absorption of products of the widespread almost simultaneous damage to epithelial cells. It is not due to any generalization of the virus through the blood.

The rapid march of an influenza epidemic through the community must also be directly based on this characteristic pathology. In each infected individual, virus liberated from damaged cells of the respiratory epithelium will reach the nasopharyngeal region from the nasal cavities or from the trachea and bronchi and will contaminate the saliva. Transfer of infection to others is by liberation of droplets of infected saliva into the air. In most cases the virus is probably inhaled in the form of the minute "nuclei" resulting from the evaporation of these droplets. The virus may lodge on any region of the respiratory epithelium and initiate a spreading surface infection from its point of primary lodgement.

In a typical influenza epidemic where a community containing a large proportion of susceptible people is attacked by an influenza virus of high or moderate virulence, the sequence of events is more or less what would be expected on the simplest epidemiological reasoning. The infection has a very short, two-day, incubation period; large amounts of virus are liberated into the saliva and thence into the air as droplet nuclei. The infection, therefore, spreads rapidly, but as infection is followed by a solid —if temporary—immunity, the number of available susceptibles is rapidly reduced, and the epidemic disappears within a few weeks.

In these lectures, however, we are concerned not so much with the character of epidemics as with the means by which the virus can survive between epidemics, and with the conditions which determine the initiation of a new epidemic. It is probable that much more will need to be known about the influenza-like ailments not due to viruses A or B before it will be possible to give an adequate account of the ecology of the influenza viruses. All that can be attempted is to discuss some of those advances in knowledge which will require to be fitted into the final picture, and to indicate some of the more important gaps.

Certain general characteristics of the minor respiratory infections may first be mentioned. These include true virus influenza as well as a proportion of primary bacterial infections and a large residuum, colds and febrile catarrhs, of undetermined etiology. The clearest idea as to the basic nature of the interaction between man and this group of infective agents is probably to be drawn from their behaviour in semi-isolated communities. Arctic communities have provided a considerable fund of material for this purpose, notably communities in Spitsbergen (104) and Greenland.

In Spitsbergen before the war the population consisted mainly of Norwegian coal miners living in a few separated mining settlements. They were completely isolated from the outside world from November to May, and during this period the population was free from respiratory infection except for a few sporadic colds. Such colds as occurred were associated particularly with visits in the spring from persons of one mining settlement to another. Abs, quoted by Høygaard, states that definite epidemics of colds might be produced in this way *before* the arrival of the first ship of the summer season. With the arrival of the first ship there was invariably an explosive epidemic of colds, including a proportion of febrile attacks. Colds continued fairly prevalent till September, after which they gradually disappeared.

In Angmagssalik, a very isolated Eskimo community in East Greenland, served by only one ship a year, conditions are similar to those in Spitsbergen. In the spring, before the ship arrives, meetings of people from isolated settlements produce mild outbreaks of colds, but a much more severe outbreak follows the ship's arrival in the summer. At irregular intervals, influenza appears at this time, the last reported instance being in 1935. This epidemic began two months after the ship's arrival; it spread rapidly along travelling routes but did not reach communities cut off from all contact with infected centres. There was a high mortality, involving 10 per cent of the total population, so that one can feel reasonably confident that the disease was true virus influenza associated with some virulent bacterial concomitant.

Another similar example was the epidemic of influenza in Point Barrow, Alaska, in 1935. This commenced 8 days after the arrival of three apparently healthy travellers by plane from Canada. It is distinguished from dozens of similar episodes only by the circumstance that influenza virus A was isolated from sputa sent to the United States (105).

From such occurrences, several deductions can be drawn which appear to be in accord also with the less easily analysed behaviour of respiratory infections in civilized communities. First, resistance to infection is due to recent past infection with the virus concerned. Second, in the "colds" group of infections, resistance is rather highly specific, people immune to the viruses of their own community being still sensitive to those of another isolated group. Third, asymptomatic carriers of colds viruses are extremely common amongst persons who have recently left large civilized communities. Fourth, even in summer a (presumably much smaller) proportion of such persons carry influenza virus.

In civilized urban communities respiratory diseases are ubiquitous, yet it is only for relatively short periods that any one type is epidemic, and even in epidemic periods most persons escape obvious infection. The importance of repeated past experience in determining resistance can be seen on many occasions, particularly in military communities. In camps and barracks it is invariably noted that recruits, particularly recruits from country districts, suffer most from influenza and other respiratory infections. Stuart-Harris and his collaborators found this very evident in a naval establishment in England which they studied during the influenza A epidemic of 1937 (106). Amongst trained men with more than a year's service the incidence of influenza was 3.8 per cent; amongst recruits of the same age, 12.7 per cent. In these figures and others from similar sources there is a suggestion that life in crowded communities may produce a certain non-specific toughness against symptomatic influenza. Unless influenza A was more prevalent in England between 1933 and 1937 than the records indicate, it is hard to ascribe the resistance of seasoned men wholly to their more numerous contacts with the specific virus. An episode in our experience (107) speaks for the possibility that this non-specific resistance manifests itself not by preventing infection but by increasing the proportion of subclinical to clinical infections. In a typical influenza A epidemic in July, 1939, we studied two populations—one, the laboratory staff; the second, a ward of 72 mental-hospital inmates. Clinical and serological studies showed the following incidence of clinical and subclinical infection: in the laboratory staff 8 clinical cases, 4 subclinical, with 21 showing no evidence of infection; in the mental hospital, 15 cases, 45 subclinical infections, and only 12 without antibody rise. The difference is striking, but its interpretation along these lines can only be tentative. The very big proportion of subclinical infections in the second group may have been due to non-specific resistance induced by constant interchange of respiratory pathogens, but the greater age and the mental condition of these patients may also have played a part.

On a larger scale, the most conspicuous feature of the epidemiology of influenza A since 1933 has been the absence anywhere of extensive influenza epidemics in two consecutive winters. The intervals between major epidemics have been in America 4–2–2–3 years, in England 4–7 years, and in Australia 4–3 years. After passing through a widespread epidemic a community acquires a resistance which for 1 to 3 years prevents the initiation and spread of a similar epidemic.

From the wealth of available data, it is difficult to decide what aspects of influenza best exemplify the necessity for the ecological approach. The most important manifestation of influenza, the pandemic of 1918–19, provides a number of outstanding problems which have been discussed elsewhere (108), but in the complete absence of knowledge as to the character of the virus or viruses responsible it is best left out of present consideration. As far as present-day influenza is concerned, the three topics most relevant to the general theme are, first, the means by which the virus survives between epidemics; second, the variability of influenza virus; and third, the influence of immunity in determining the manifestations of influenza and the possibility of preventing the disease by immunological measures.

THE CARRIER STATE IN INFLUENZA

Very little can be said about the frequency of influenza virus carriers or the form in which the virus is carried. No systematic search for carriers has been reported, and it seems highly improbable that present methods for the isolation of virus would be of any value for such a study. The mode in which the virus persists in the hypothetical carrier can only be a matter for speculation. By analogy with herpes we might picture a group of respiratory cells latently infected by virus which is only called into activity by some appropriate stimulus, perhaps a cold or some particular climatic state. Until some new approach to the problem develops, it seems unlikely that any evidence other than epidemiological will be available on the carrier question. All that can be said is that at the present time there is no visible alternative to the view that human influenza viruses survive between epidemic periods in the tissues of human carriers.

In the case of the related disease, swine influenza, Shope (109) has described a totally different means by which the virus survives. This involves the entry of the virus into the parasitic lungworms of the pig, and its survival for periods up to two years in the earthworm, which is the intermediate host of the larval lungworms. When, after the infested earthworms are eaten by pigs, the lungworm larvae pass to the lungs, the virus initiates a latent infection. The typical acute infection can only be provoked by some as yet indeterminate stimulus, which in the field is associated with the first spell of wet cold weather in October or November. There are some aspects of this life cycle which are still matters of inference rather than of demonstration, but there seems no reason to doubt that it is essentially correct. Shope's work at once opens up a great many interesting questions. If our general contention is correct, that human epidemic diseases are with few exceptions derived eventually from the diseases of animal communities, the pig becomes the most likely source of the influenza viruses. There is no evidence as to whether or not swine influenza affects wild pigs, or if so whether the earthworm serves as an intermediate host. It is of interest, however, that a cycle of the type described by Shope would make it possible for swine influenza virus to survive in populations of wild pigs living in small droves. An acute respiratory infection with no secondary reservoir would infallibly die out under such conditions. It is quite possible, therefore, that the Shope cycle may represent the most ancient mode of life of the influenza viruses. But there is equally no valid evidence that it is not a mere biological accident, perhaps dating (as Shope suggested in earlier work) only from the human pandemic of 1918. Until the ecology of swine influenza in regions other than the mid-western states has been worked out in the same detail, and until it has been determined whether swine influenza and lungworm are present in wild pigs, it is premature to discuss the evolutionary history of the virus.

There are two points from Shope's work, however, which are highly relevant to our point of view. Swine influenza in Iowa has two distinct modes of transference. The primary cycle is the complex one that has been described, but once the disease has been called into activity it can spread readily, presumably by the aerial route, to other susceptible pigs. If a variant should arise capable of infecting a foreign host (man, perhaps), there is no reason whatever why it should not permanently adopt only the second method of transfer—always provided that it could find in this way a means of indefinite survival. The second point of interest is that, in or near the worm lesions in the lung, virus taken in with the larval worms can lie latent until stimulated to activity in some non-specific fashion. There is a suggestion here that in human carriers the influenza virus might similarly persist in pathologically altered cells around some chronic lesion in the upper or lower respiratory tract, and be liberated again in response to a non-specific infective or environmental stimulus.

VARIATION IN INFLUENZA VIRUS

The outstanding characteristic of the influenza viruses is their mutability. Even amongst the strains isolated from a single epidemic of influenza A, minor but distinct antigenic differences can be recognised. There may be wider differences still when strains from different epidemics are concerned. The strain "W.S." isolated in England in 1933 is strikingly different in antigenic structure from any of the strains obtained in America or Australia since 1935. The ease with which strains from human throats can be adapted to laboratory animals varies from epidemic to epidemic, and sometimes within the same epidemic. As a rule, isolation is easy when there is a sharp typical epidemic of influenza A, more difficult when there are only minor prevalences. These differences in influenza strains as they are isolated are only indirect evidence of current mutability in the virus. Each strain might conceivably be derived only from strains of similar character, there being perhaps the same multiplicity of true-breeding minor types as we find, for instance, amongst the haemolytic streptococci.

Laboratory study of individual strains, however, gives direct evidence of the great lability of the virus. There is, in fact, more detailed information available about variation in influenza viruses than there is for any other type of animal virus. For the reasons given earlier, a knowledge of the nature and limits of variation in pathogenic micro-organisms is indispensable for the understanding of the phenomena of infectious disease. This is particularly so when we are concerned with the transfer of infection from one species to another. It may be worth while, therefore, to give an account of some of the work we have carried out in Melbourne on the changes that influenza virus A strains undergo in the process of adaptation to laboratory passage (110).

Our standard method for primary isolation of influenza virus from human throat washings is inoculation of the amniotic cavity of the developing chick embryo with the filtered material. The method was originally developed to exploit the fact that the amniotic cavity is in direct communication with the respiratory tract of the embryo, and that an unadapted pneumotropic virus might be expected to grow better there than elsewhere in the embryo. Active growth does in fact take place in the developing lung, but it is probable that growth also occurs in cells of the amniotic epithelium. Before Hirst's discovery of the haemagglutinating activity of influenza viruses, the presence of infection in the embryo was recognised by the characteristic cellular content of the tracheal fluid. The haemagglutinin content of the amniotic fluid is, however, a much more convenient test and has quite replaced the former procedure.

As soon as we began to use Hirst's method for the purpose, it became obvious that fowl cells were very poorly agglutinated by positive amniotic fluids of the first generation. Other types of cell were tested, and both human and guinea pig cells were found to be agglutinated by such primary fluids to much higher titres than fowl cells. With our modifications of Hirst's titration method the titres might be with fowl cells 15, with guinea pig cells 160; and the character of the virus was described by saying it had a F/G ratio of 15/160. On the next passage, using a 1:10 dilution of amniotic fluid, the F/G ratio of the amniotic fluid obtained is close to 1, say 160/240, and in all subsequent passages a high F/G ratio is the rule.

But if the first-passage fluid is serially diluted, and each dilution tested in embryos by the amniotic route, it will be found that the fluids from embryos inoculated with the highest dilutions give low F/G ratios, while those with lower dilutions give a ratio of about unity. As a rule there are no intermediate fluids. This result of titration passage could be continued for a variable number of generations, twelve being the longest series we have obtained. We have called the virus which gives a low F/G ratio the original or O phase, since it is in this form that the virus is isolated from the patient. The type showing F/G values near unity or above it is known as the derivative or D phase. All stock strains propagated in mice or in the allantoic cavity are in this phase. Further study made it clear that O-phase virus will not grow in the allantoic cavity and probably fails completely to infect mice intranasally. The D phase grows to full titre in the allantoic cavity, and although it produces initially only slight lesions in mice will immunize them in small dose against a mouse-adapted strain of the same antigenic type.

The O–D change is of interest from two points of view. In the first place it represents a formal demonstration that virus adaptation to a new host is due to the selective survival of spontaneously occurring discontinuous mutants. The change is *not* due to a direct modifying action of the new host tissues; we have O virus which has spent more than a month in living chick cells without undergoing change, and we have D-phase virus obtained after a single passage. A rough estimate (which could be made accurate by suitable quantitative experiments) indicates that of every 10^5 to 10^6 particles produced by the multiplication of O virus one is in the D phase, and that, once it has appeared, D-phase virus multiplies in the amniotic cavity at about twice the speed of the O phase.

The second point of importance is that all the influenza A strains we have isolated have been in the O phase, yet all laboratory strains in use for the study of virus character or for the preparation of vaccines are in the sharply distinct D phase. It is reasonable to think that the cause of influenza A is the O phase of the virus, and one has an uneasy feeling that perhaps all our current influenza laboratory work is being done with something which has ceased to be the pathogenic virus with which we ought to be primarily concerned. Little progress would have been made in dealing with pneumococcal pneumonia if only rough pneumococci had been available for study, and pertussis immunization only became effective with the clear definition of phase I organisms.

There are a number of other interesting aspects of influenza virus A variations, but none are of the same importance as the O–D change. A D strain which has had only a few egg or mouse passages can still produce moderately severe symptoms in human beings, but there is no evidence as to whether the disease produced is or is not transmissible by the natural process to others. In all probability it lacks this quality, the typical highly infectious character for man being confined to the O phase. With continued amniotic or allantoic passage the human virulence falls, and with recent strains about 40 passages will reduce the virulence sufficiently to make the living virus a practical immunizing agent. In some instances, at least, further passage will reduce the virulence so greatly that neither symptoms nor immunizing effect is produced in susceptible subjects sprayed intranasally with the virus.

There is one other type of influenza virus variation in the laboratory which is of some general interest. This is the development by continued passage on the chorio-allantois of highly virulent endotheliotropic strains which kill the embryo with gross haemorrhagic lesions within 48 hours or less. We have studied chiefly the strain "Melbourne," but a similar variant can be obtained even more readily from the classical strain "W.S." (111, 112). Equally prolonged passage of three other standard strains, "Swine 15," "BUR" (Andrewes and Wilson Smith), and "Lee B," failed to provoke any appearance of such a variant. When "W.S." or "Melbourne" was transferred for 100 passages by the *amniotic* instead of by the chorio-allantoic route no more than trivial changes occurred. It seems that for a gross mutation to occur, such as that to the haemorrhagic form, a series of step-like mutations must lead up to the genetic stage from which the final mutant can appear. The chorio-allantois is apparently a more alien environment than the inner cavities of the embryo and has a more actively selective effect on such mutants as arise.

Our "M.E." (Melbourne egg) strain differs from a normal laboratory strain of influenza A virus in several respects. It produces relatively large, well-defined foci on the chorio-allantois and kills the embryo within 48 hours. The distribution of the haemorrhagic lesions varies somewhat with the age of the embryo and according to the degree of virulence that the virus has reached. The most striking lesion is an intense haemorrhagic encephalitis which leaves the brain a diffuse purple mass distending the skull. The strain grows very poorly in the allantoic cavity, usually failing to give enough virus in the fluid to agglutinate red cells. When allantoic or amniotic fluids of sufficient activity are obtained, an aberrant type of haemagglutination is observed. Like an unadapted "human" strain, it gives O-type agglutination with a titre against guinea pig cells 3 to 5 times as high as against chicken cells (113). As might be expected from the generalized haemorrhagic lesions which follow inoculation of the membrane, the strain produces fatal infection to limiting titre when inoculated intravenously in the chick embryo. Normal influenza strains are innocuous by this route. Finally, the strain is completely avirulent for ferrets, mice, and human beings, but immunizes the first two species against virulent strains of the same immunological type.

In many ways this "M.E." strain is a mere laboratory curiosity, but it does indicate the extraordinary lability of certain strains of influenza virus, and in a concentrated artificial way suggests how under some exceptional natural circumstances a virus normally parasitic on one host might give rise to a disease of very different character in another.

Experience with the "M.E." strain, too, led us to a quite unexpected observation which may bear closely on the appearance of "new" types of influenza virus. The virus of "Newcastle disease," a highly fatal infection of chickens widespread in the Far East, produces chick embryo lesions almost identical with those of "M.E." influenza virus (114). Large amounts of virus are found in the embryonic fluid after appropriate inoculation, and these fluids can agglutinate chicken red cells much as influenza virus does. The virus is immunologically quite distinct from any available influenza virus, but it can be shown that it becomes attached to the same "receptors" on the red cell surface as take up influenza virus. Further, the size of the virus particles is very close to that of the influenza virus (60–100 $m\mu$), and with high titre virus an influenza-like consolidation of mouse lungs can be produced. Finally, a laboratory accident showed that Newcastle disease virus could produce a severe conjunctivitis in a human being with brief febrile symptoms and the appearance of antibody in the blood (115).

We concluded that these resemblances could only mean that the two groups of viruses were related and derived eventually from a common ancestral form. Fowl plague virus is also known to agglutinate red cells and to produce rather similar lesions in embryos. It also may be a member of the influenza group of viruses.

From the human standpoint, the ancestry of Newcastle disease virus is of less importance than the possibility that it or some similar virus might on occasion find opportunity for human passage and by the appearance and selective survival of appropriate mutants give rise to a new antigenic type of influenza. Our experience with "M.E." and other strains of influenza virus modified by laboratory manipulation leads to the somewhat disturbing conclusion that within the virus of Newcastle disease, and perhaps of a dozen other obscure respiratory viruses of animals, there is a potentiality of becoming a full-blown human pathogen.

Without looking to the remote possibility of the emergence of new viruses, these studies of variation in influenza viruses demonstrate at least the highly labile character of the organisms. It is easy to see how, with rapid passage (i.e., with the development of an enormous population within which mutants can arise), variants, on the one hand, of high virulence and, on the other, of capacity to lie latent in tissues will be more likely to appear. Both types may have temporary or permanent survival value for the species.

THE SIGNIFICANCE OF IMMUNITY IN INFLUENZA

When one endeavours to understand the epidemiology of influenza in the light of laboratory work on the viruses, one cannot help being struck by the fact that even in the greatest epidemics so many people are *not* affected. In England, during the severe autumn wave of the 1918 pandemic, of any 1000 people about 800 escaped clinical infection, 180 had an ordinary 3- to 5-day attack of influenza, and only 20 had pneumonic complications from which 5–10 died (116). Individual groups in this and other epidemics showed much higher morbidity, but in no large community does the morbidity ever approach 50 per cent.

The main practical objective of influenza research might be said to be, to find out why the majority of individuals resist influenza during an epidemic, and on the basis of that knowledge to devise measures to confer the same advantage on susceptibles.

Very extensive studies have been made to determine whether one or other of the methods of detecting influenza antibody in the blood can provide data which will separate susceptibles from non-susceptibles. A fair statement of the results obtained is that in natural epidemics there is some positive correlation between the occurrence of clinical infection and a low antibody level. In artificial tests for immunity, using large doses of more or less attenuated D-phase virus, there is a much higher degree of correlation between clinical symptoms and a low antibody level. The general impression one gains is that antibody level as tested, e.g., by Hirst's haemagglutination method, is merely an index of past infection more or less definitely correlated with some other more effective immunological mechanism. It is still to be determined what that mechanism is.

In our laboratory, since 1936, we have been working on various methods of estimating influenza virus antibody; at various times we have used the mouse-neutralization test, neutralization of egg-adapted virus on the chorio-allantois, the complement-fixation test, Hirst's haemagglutination method, and, most recently, neutralization in the allantoic cavity of the chick embryo. In addition we have developed a skin test for the low-grade allergic reactivity associated with past infection. No two of these tests give fully correlated findings with human sera. With ferret sera there is in general a good correlation, a recently infected animal showing high titres by all tests; but there are no reports of the comparative titres in sera from ferrets which have been kept for a long period since immunization or, like most adult human beings, reinfected with different strains at irregular intervals. If one were dealing with human populations which were infected with influenza viruses only at intervals of many years, antibody titrations might give a clear indication of the past history and present immunity of each individual, as, for example, yellow fever antibody titrations can. But the problem is much more complex. Influenza B seems to be endemic most winters in most countries, usually without producing epidemics. Influenza A epidemics appear at least once every four or five years, often every second year, and there are probably always some carriers liberating virus in any large community.

The immunological status of any individual is, therefore, constantly changing, and at any given period the circulating antibody is the resultant of many different antigenic stimuli, the antibody molecules differing in their antigenic type and in their avidity as well as in their actual concentration in the blood. The result observed will certainly depend both on individual physiological factors and on the experience of exposure to influenza viruses in the past.

If a clear picture of the immunity response to influenza is to be obtained it will be necessary to start systematically and study first the antibody response in children suffering their initial experience of the virus. Very little systematic work has been done with children's sera, but there are plenty of hints that such an investigation would pay handsome dividends. Very early in the story of influenza research the Hampstead group found that in children under 12 there was frequently antibody against the "W.S." strain of influenza A, but none against swine type virus (117). This was first interpreted as perhaps indicating that pandemic influenza was of swine antigenic type, but the interpretation was dropped when it was shown that following infection in adults with an A strain both "W.S." and swine type antibodies showed a sharp rise. In 1937 Miss Lush and I showed that influenza A antibody in children's sera was specific in that it neutralized the presumed infecting sub-type ("Melbourne") and was inactive against both "W.S." and swine sub-types (118). In fact, the sera behaved very similarly to those obtained from experimentally infected ferrets. We have had no recent opportunity to continue this work and there has been no similar work reported elsewhere. From the point of view of theoretical immunology it is a very interesting phenomenon that a child produces a highly specific type of antibody, while an adult exposed to the same stimulus produces one of much broader activity. Beveridge and I (33) have recently uncovered a possibly related phenomenon in working with an intradermal test. Boiled allantoic fluid infected with A or B virus is used at a suitable dilution, 0.1 cc. being injected intradermally, and the reaction read 24 to 30 hours later. As a control we used a similar preparation from Newcastle disease virus. In children it was found that reactions were weak, requiring the use of undiluted fluid to give easily measurable positive reactions. Positive reactions were always associated with antibody of the corresponding type in the blood, A and B reactions being quite independent. A considerable number of children with circulating antibody, however, failed to show the corresponding skin reaction. In adults the conditions were quite different; nearly all subjects showed reactions to both A and B, and about 30 per cent also reacted to the Newcastle disease virus preparation. The reactions were sometimes large and distinct, even with 1:10 dilution of the reagents. As is found in many such phenomena, the adult reaction is more severe and immunologically broader than the child's.

To return to the question of immunity in the adult: it should be remembered that, if they are to survive, the influenza viruses must have some capacity at least to multiply in the respiratory epithelium of persons with a basic immunity from past infections, which in practice means every adult in an urban community. It is probable that this capacity is related to the fact that influenza is essentially a surface infection, its spread from cell to cell requiring no passage through blood or tissue fluids. If, as we must assume, antibody is a protective mechanism against influenza virus infection, it can only come into play in any force when secondary inflammatory changes are produced as a result of the primary necrosis. The faster the spread of infection over the surface, the less effect circulating antibody will be likely to exert; conversely, the lower the virulence, the more obvious the effect of antibody will be. This probably explains why in all the reported experimental human infections with laboratory strains of virus there has been a much closer correlation of symptoms with low antibody level than is observed in an epidemic.

The most popular approach toward a method of preventing influenza is to give subcutaneous inoculations of killed virus, usually in the form of allantoic fluid suitably prepared. There is no doubt that by this means a sharp rise in circulating antibody can be induced in most subjects, and that the antibody production is correlated with the amount of antigen injected. It is, therefore, at least theoretically possible to reach the maximal level of antibody level of which the subject is capable by using concentrated virus vaccines. The results obtained in such a test during the American epidemic of November and December, 1943 (119), were at first sight very encouraging. The preliminary tabulation shows that of some 6200 immunized subjects, 138 (2.22%) contracted clinical influenza as against 442 (7.11%) in a similar number of control subjects; i.e., there were 3.2 cases amongst the controls for every case in the immunized group. A retabulation of the figures according to the time elapsing since immunization shows, however, that the benefit obtained was only strikingly evident in the period from 2 to 4 weeks after immunization. This brief duration of apparent immunity may be susceptible to more than one explanation. It is not related to any obvious fall in antibody titre, though the possibility of a qualitative deterioration in antibody must be considered. It may, as Francis has suggested, be related more to the phase of the influenza epidemic than to the period elapsing after immunization.

This work of the United States Army Commission on Influenza is the most promising approach yet made toward the prevention of influenza, but it would be premature to regard the problem as solved. A success gained, perhaps, by fortunate timing of immunization just before the appearance of a widespread but mild epidemic does not necessarily mean that the same methods would be applicable on a practical scale against severe influenza of pandemic type. In any epidemic period, the problem of immunization is essentially to protect the proportion of people—usually a relatively small proportion—who would otherwise contract symptomatic infection. We do not know what factors determine this apparent division of the population into susceptibles and non-susceptibles. We can feel fairly sure that these factors will vary from one epidemic or community to another. It may well be that quite uncontrollable factors largely determine whether or not an individual is infected symptomatically—the size of the infecting dose, the intrinsic virulence of the virus and the degree of partial attenuation it has undergone in transfer, and perhaps temporary physiological conditions of the respiratory mucosa may all play a part. For a large group of persons of borderline immunological status, these factors may determine whether first exposure to the virus during an epidemic results in clinical or subclinical infection. On either side of this group, the immunological evidence would suggest that there are (a) persons whose immune response to past infection has left them with a specific resistance (due either to antibody level alone or to other factors in addition) which ensures protection against the virus under any normal circumstance, and (b) other individuals whose specific immunity is so low that they are certain to be infected clinically by first effective contact with the virus. Within the susceptible group (b) would be found those persons who have not been exposed to infection for long periods and have lost any immunity they formerly possessed, as well as those who have been exposed to the recurrent normal chances of influenza but have failed to develop or maintain normal immunity. It is reasonably certain that these groups must exist, but there is little to indicate what proportion of a normal urban population falls into each group, and, short of very extensive controlled human experimentation, there is no obvious means of determining these proportions.

General epidemiological experience would suggest that the first-mentioned group of people, whose susceptibility or resistance is determined essentially by the circumstances of their exposure to the virus, is of dominating importance. On this hypothesis, the possibility of effective action in diminishing the incidence of influenza is made much more likely than if susceptibility or resistance is more rigidly determined by the effectiveness or otherwise of the immunity mechanism at the time of exposure. In particular, it offers a definite hope that means of diminishing the transfer of infection from person to person, even if only partly effective, might produce a relatively large shift from clinical to subclinical infections. Immunization might be expected to act not only by protecting a proportion of the immunized individuals but also by diminishing the infectivity of persons who do contract infection despite immunization.

Every comprehensive study of human antibody response to an immunizing agent uncovers a proportion of individuals who develop little antibody or lose their maximal level abnormally rapidly. Jensen's work on the persistence of antitoxic immunity after immunization against diphtheria is a typical example. Amongst such persons, effective immunization against influenza will probably be impossible. On the other hand, it might well be particularly effective in persons whose low resistance is due only to absence of any recent contact with the virus.

If Francis's view is correct, that the mode by which antibody protects against influenza is the liberation of a small proportion into the fluid bathing the respiratory epithelium, another source of individual variation can be recognised. This is in regard to the amount and rapidity of liberation of antibody on the respiratory mucosa. A person with a capacity for rapid liberation, perhaps with an essentially allergic basis, might be better protected than another with an equal level of circulating antibody but with a less efficient mechanism for mobilizing it.

With this possibility in mind, we have spent much time in Melbourne in endeavouring to develop a method of immunization against influenza by the use of attenuated living virus (120). This has been shown to be technically practicable in the sense that both A and B strains can be obtained which can be administered intranasally to large numbers of subjects without the production of symptomatic influenza. A proportion ranging from 15 to 60 per cent of those so treated show a significant rise in antibody as a result. They also show a sharp increase in resistance to the antibody-provoking effect of a second intranasal spray given 4 to 6 months later. In the light of subsequent experience, we are more than doubtful whether this can be taken as representing an equivalent resistance to symptomatic infection by a natural strain. So far, only one unsatisfactory opportunity to gauge the value of the method in the face of an influenza A epidemic has arisen. The results obtained did not suggest that the intranasal administration had had more than a trivial effect on the incidence of influenza. The activity of the vaccine strains has been improved since this test, and further experimental trials are in progress.

Theoretically, there are both advantages and disadvantages in using the nasal route; and on the practical side there is the advantage of ease of preparation and administration on a large scale to be weighed against the possible dangers associated with use of a living virus.

If it were a question of providing a primary immunity against influenza in a population not previously exposed to it, the advantages of the intranasal method, with a suitably attenuated strain, would probably be outstanding. Natural immunization against influenza is obviously a highly effective process, and the intranasal method is the closest possible imitation of natural conditions. But the practical problem—protection of service personnel or other young adults—is a different matter. Here what is required is to reinforce a basic immunity in those individuals who have failed to develop an adequate resistance as a result of their natural contacts with the virus. The results of the United States Army Commission must be accepted as proving that under favourable circumstances this can be done by the subcutaneous inoculation of concentrated vaccine. Future developments will probably be directed toward prolonging the antibody rise, perhaps by the use of precipitating agents like alum or protamine in the vaccine. It is still possible that a combination of living attenuated virus intranasally with the subcutaneous inoculation might be valuable in increasing the resistance of those subjects who have escaped contact with the virus for some years.

It seems particularly inadvisable to assume on the basis of a single success against a relatively mild epidemic of influenza A that a means of dealing with a major influenza pandemic is available. The antigenic type of the virus responsible for the pandemic of 1918 and the circumstances which endowed it with such extreme virulence are both completely unknown. Judging from the high mortality, the recrudescence of successive epidemic waves, and the incidence of death in the young adult age group, it is highly improbable that the vaccination of our current type would have had any significant effect. This is on the most reasonable assumption, that the pandemic was due to the spread of hypervirulent strains of influenza A virus. If the virus responsible were antigenically of a new type—if, for instance, the 1918 pandemic represented the first appearance of influenza A virus as a human pathogen—then appropriate vaccination, if suitable preparations could have been made in time, might conceivably have reduced the character of the pandemic to that of ordinary influenza.

If another similar pandemic should arise in the near future, the major task of virus research workers should be to obtain the fullest possible information in regard to the origin and spread of the epidemic, and especially to determine the characteristics and range of variation of the virus strains responsible. Public pressure will force health authorities to provide some form of panacea, and it is only too likely that most workers on the subject will be obliged to fritter away their activities making and administering vaccine of one sort or another. The one great feature of influenza which has not been manifested in the period of effective research is the major pandemic. Until the natural history of such an episode has been elucidated it will be impossible either to understand the whole influenza situation or to devise measures to minimize the effect of a subsequent pandemic.

X

CONCLUSION

IN THE PRECEDING chapters I have tried to discuss some of the more important virus diseases of man from a consistently biological point of view. Each of these diseases has its own individuality, and as far as practical measures are concerned, each must be studied in detail as an individual problem. It is a matter for argument whether or not a scientific worker has any justification for attempting to go beyond the accumulation of accurate and relevant facts bearing on some problem of human importance. But in infectious disease, as in most other biological fields, the infinite complexity of the facts makes some attempt to co-ordinate them to a common theme essential. The public health administrator, for instance, must have some broad working knowledge of infectious disease as well as access to all the relevant details of any specific problem. A discussion of some viruses responsible for human disease around the central theme of their primary biological attributes—reproduction, variation, survival and evolution—can give only a very incomplete account of the facts; but it does, I hope, provide a valuable line of approach toward any new aspect of virus disease which may present a practical problem of control.

It is difficult to attempt any sort of general summary beyond reiterating the basic interpretation of virus disease that we have adopted. This is, in brief, that viruses are micro-organisms which have evolved by parasitic degeneration from larger micro-organisms, many of them in all probability from bacteria. They still show evidence in their chemical structure of conformity with the general pattern of living material, and their behaviour must be interpreted mainly in terms of biological concepts.

Viruses reproduce by replicating their structure at the expense of suitable atomic groups within the living substance of a susceptible cell. No alternative description of this replication has been able to replace the view that it is a simple example of biological reproduction. Viruses can vary, and the variant quality can be transmitted to descendants. Some examples have been given to show the striking differences that may exist between original and variant strains of virus. There is every reason to believe that variants arise by a process analogous to gene mutation in higher forms and that they become demonstrable only when the environment is such as to favour the survival of the variant over the original form. Finally, we have stressed the necessity that a virus species must, like any other living species, have found a way of indefinite survival. This mode by which the species survives may have little immediate relationship to the obvious clinical manifestation of the virus's activity.

Perhaps a little more may be said on the broader evolutionary aspect of virus disease. We have stressed throughout the importance of the transfer of virus infection from one host to another, and have given examples to show that such transfers from animal reservoirs to man are taking place at present; we have also postulated that in the past all or nearly all the known virus diseases of man must have been similarly derived from animal infections. The process by which such transfer to new hosts can, by the selective survival of suitable mutants, result in a new mode of indefinite survival for the virus species has also been discussed. Irrespective of whether attention is directed to the large-scale epidemic manifestations of virus disease or to the phenomena encountered in the course of laboratory manipulation, one receives a particular impression of the role played by pure chance in determining the pattern of virus disease, as of infectious disease in general. Parasitic micro-organisms are genetically labile, viruses perhaps more so than others. They are frequently subject to alternate phases of excessive proliferation and cataclysmic destruction. Under such circumstances selection for survival is intense, but it is a blind selection for immediate survival only. If a single surviving variant of appropriate character arising within the population of a pathogenic micro-organism finds the one-in-a-million chance of initiating infection either in the normal host or in some other species, it may within a short period produce a new dominant type of disease. This is what seems to have happened in 1857 when modern type diphtheria made its appearance and within two or three years swept round the globe.

For those who look for progress and design in evolution, the picture that has been built up of the evolution and natural history of virus disease must be an almost intolerable one. For the viruses there has been no steady increase in complexity and emergence of new powers but, as they have evolved, an increasing simplicity of structure and a greater dependence on the host cell for nutrition, until in the smaller viruses there are neither organs nor enzymes, nothing but a shadowy, self-replicating residuum of genetic mechanism.

Viruses as a group play no part in the major biological cycles that maintain an uneasy status quo on the earth's surface. At most they act as one of the agents by which excessive proliferation of bird or mammal is countered. Like predators and natural calamities, they are part of the inescapable environment that every surviving species has had to come to terms with. Even their periodic exacerbations of virulence have an irrational quality. Excessive virulence serves no useful purpose in the survival of the species; it can in the last analysis be regarded only as an accidental result of minor change in the genetic pattern of the species. In higher forms there is an elaborate mechanism for "buffering" the effect of a mutation in the individuals carrying the changed genes. In viruses no such buffering can be thought of. When an excessively virulent mutant appears and survives the initial hazards of transfer to a small number of susceptible host individuals, an abnormally severe epidemic will result which will end normally with extinction of the aberrant virus strain. More rarely, a permanently changed situation will be established; for example, by a lasting increase in the proportion of overt infections produced in the host species.

The unpredictable character of changes in virulence for the same and for foreign hosts makes it impossible to forecast the future of such diseases as the respiratory virus infections which it is impossible to control by appropriate protective measures, or to suggest from what sources new virus diseases of human importance may be derived. In view of the extensive natural reservoirs of viruses in arthropod, bird, and mammal that have been uncovered by investigations into disease of man and domestic animals, it is only natural to believe that many others may be discovered. From these, by some suitable combination of chance and circumstance, new virus diseases of man may well arise in the future.

Control of virus disease in the future, as in the present, must depend wholly on an accurate understanding both of the mode by which the virus survives and of the process by which symptomatic disease is produced. When such knowledge is available, it is usually possible to see either that effective action can be taken at some point in the life history of the virus to eliminate the chance of human infection or that the virus cannot be dealt with in this fashion. In the latter case, the aim changes to that of preventing symptomatic infection after the virus has entered the body either by ensuring an immunological resistance through artificial immunization carried out before exposure to the virus or by other means still to be devised.

Big future developments in the finer laboratory study of viruses can be looked forward to with certainty. With further elucidation of their chemical structure, of their mode of using the enzyme systems of the host, and of the physical chemistry of the replication process, new conceptions of the relationship of viruses to other biological units may arise. For some purposes it may be desirable to allocate them to a new category sharply differentiated from all other living organisms, but as far as the doctor, the public health administrator, and the biological experimenter are concerned, the pragmatic necessity will remain that virus be regarded as organism—self-reproducing, varying, and surviving like any other living being.

BIBLIOGRAPHY

1. Stanley, W. M.: Chemical and physical properties of viruses. *Handbuch d. Virusforschung,* **1,** 498 (Wien, 1938).
2. Elford, W. J.: The sizes of viruses and bacteriophages, and methods for their determination. *Handbuch d. Virusforschung,* **1,** 126 (Wien, 1938).
3. Smadel, J. E., and Hoagland, C. L.: Elementary bodies of vaccinia. *Bact. Reviews,* **6,** 79 (1942).
4. Doerr, R.: Die aktuelle Problematik der Virusforschung. *Handbuch d. Virusforschung,* **1,** 21 (Wien, 1938).
5. Burnet, F. M.: The production of antibodies. *Monographs from Walter & Eliza Hall Institute,* No. 1 (Melbourne, 1940).
6. Huxley, J. S.: *Evolution: the Modern Synthesis* (Cambridge, 1942).
7. Massini, R.: Ueber einen in biologischer Beziehung interessanten Kolistamm (Bacterium coli mutabile). Ein Beitrag zur Variation bei Bakterien. *Arch. Hyg.,* **61,** 250 (1907).
8. Lewis, I. M.: Bacterial variation with special reference to behavior of some mutable strains of colon bacteria in synthetic media. *J. Bact.,* **28,** 619 (1934).
9. De Kruif, P. H.: Dissociation of microbic species: I. Coexistence of individuals of different degrees of virulence in cultures of the bacillus of rabbit septicemia. *J. Exp. Med.,* **33,** 773 (1921).
10. Arkwright, J. A.: Variation in bacteria in relation to agglutination both by salts and by specific serum. *J. Path. & Bact.,* **24,** 36 (1921).
11. Burnet, F. M.: Bacteriophage activity and the antigenic structure of bacteria. *J. Path. & Bact.,* **33,** 647 (1930).
12. Luria, S. E., and Delbrück, M.: Mutations of bacteria from virus sensitivity to virus resistance. *Genetics,* **28,** 491 (1943).
13. Burnet, F. M., and Lush, D.: Induced lysogenicity and mutation of bacteriophage within lysogenic bacteria. *Austral. J. Exp. Biol.,* **14,** 27 (1936).
14. Avery, O. T., MacLeod, C. M., and McCarty, M.: Studies on the chemical nature of the substance inducing transformation of pneumococcal types. *J. Exp. Med.,* **79,** 137 (1944).
15. Beadle, G. W., and Tatum, E. L.: Genetic control of biochemical reactions in Neurospora. *Proc. Nat. Acad. Sc.,* **27,** 499 (1941).
16. DeMonbreun, W. A., and Goodpasture, E. W.: Infectious oral papillomatosis of dogs. *Am. J. Path.,* **8,** 43 (1932).
17. Laidlaw, P. P., and Elford, W. J.: A new group of filterable organisms. *Proc. Roy. Soc. London, Ser. B.,* **120,** 292 (1936).
18. Sabin, A. B.: The filtrable micro-organisms of the pleuropneumonia group. *Bact. Reviews,* **5,** 1 (1941).
19. Glaser, R. W.: The intracellular "symbionts" and the "rickettsiae." *Arch. Path.,* **9,** 71, 557 (1930).

20. Tatum, E. L.: Nutrition, genetics, and "*Neurospora*." *Stanford Med. Bull.*, **2**, 1 (1944).
21. Hirst, G. K.: The agglutination of red cells by allantoic fluid of chick embryos infected with influenza virus. *Science*, **94**, 22 (1941).
22. Swan, C., Tostevin, A. L., Moore, B., Mayo, H., and Black, G. H. B.: Congenital defects in infants following infectious diseases during pregnancy. *Med. J. Australia*, **2**, 201 (1943).
23. Meyer, K. F., and Eddie, B.: Psittacosis in the native Australian budgerigars. *Proc. Soc. Exp. Biol. & Med.*, **31**, 917 (1933–34).
24. Zinsser, H.: Epidemiology and immunity in the rickettsial diseases. *Virus and Rickettsial Diseases*, p. 872 (Cambridge, Mass.: Harvard University Press, 1940).
25. Wheatland, F. T.: A fever resembling a mild form of typhus fever. *Med. J. Australia*, **1**, 261 (1926).
26. Hoskins, M.: A protective action of neurotropic against viscerotropic yellow fever virus in Macacus rhesus. *Am. J. Trop. Med.*, **15**, 675 (1935).
27. Findlay, G. M., and MacCallum, F. O.: An interference phenomenon in relation to yellow fever and other viruses. *J. Path. & Bact.*, **44**, 405 (1937).
28. Henle, W., and Henle, G.: Interference of inactive virus with the propagation of virus of influenza. *Science*, **98**, 87 (1943).
29. Schlesinger, R. W., Olitsky, P. K., and Morgan, I. M.: Observations on acquired cellular resistance to equine encephalomyelitis virus. *Proc. Soc. Exp. Biol. & Med.*, **54**, 272 (1943).
30. Francis, T., Jr., and Stuart-Harris, C. H.: Studies on the nasal histology of epidemic influenza virus infection in the ferret. *J. Exp. Med.*, **68**, 789, 813 (1938).
31. Francis, T., Jr.: The significance of nasal factors in epidemic influenza. *Problems and Trends in Virus Research*, p. 41 (University of Pennsylvania Press, 1941).
32. Nagler, F. P. O.: A specific cutaneous reaction in persons infected with the virus of herpes simplex. *J. Immun.*, **48**, 213 (1944).
33. Beveridge, W. I. B., and Burnet, F. M.: A cutaneous reaction to the influenza viruses. *Med. J. Australia*, **1**, 85 (1944).
34. Dodd, K., Johnston, L. M., and Buddingh, G. J.: Herpetic stomatitis. *J. Pediat.*, **12**, 95 (1938).
35. Burnet, F. M., and Williams, S. W.: Herpes simplex: a new point of view. *Med. J. Australia*, **1**, 637 (1939).
36. Burnet, F. M., and Lush, D.: The inactivation of herpes virus by immune sera: experiments using the chorio-allantoic membrane technique. *J. Path. & Bact.*, **48**, 275 (1939).
37. Shaffer, M. F., and Enders, J. F.: Quantitative studies on the infectivity of the virus of herpes simplex for the chorio-allantoic membrane of the chick embryo, together with observations on the inactivation of the virus by its specific antiserum. *J. Immun.*, **37**, 383 (1939).
38. Andrewes, C. H., and Carmichael, E. A.: A note on the presence of

antibodies to herpes virus in post-encephalitic and other human sera. *Lancet,* **1,** 857 (1930).

39. Teissier, P., Gastinel, P., and Reilly, J.: L'herpès expérimental humain. L'inoculabilité du virus herpétique. *J. de physiol. et de path. gén.,* **24,** 271 (1926).
40. Levine, H. D., Hoerr, S. O., and Allanson, J. C.: Vesicular pharyngitis and stomatitis: an unusual epidemic of possible herpetic origin. *J.A.M.A.,* **112,** 2020 (1939).
41. Scott, T. F. McN., Steigman, A. J., and Convey, J. H.: Acute infectious gingivostomatitis: etiology, epidemiology and clinical picture of a common disorder caused by the virus of herpes simplex. *J.A.M.A.,* **117,** 999 (1941).
42. Black, W. C.: The etiology of acute infectious gingivostomatitis (Vincent's stomatitis). *J. Pediat.,* **20,** 145 (1942).
43. Smith, M. G., Lenette, E. H., and Reames, H. R.: Isolation of the virus of herpes simplex and the demonstration of intranuclear inclusions in a case of acute encephalitis. *Am. J. Path.,* **17,** 55 (1941).
44. Zarafonetis, C. J. D., Smadel, J. E., Adams, J. W., and Haymaker, W.: Fatal herpes simplex encephalitis in man. *Am. J. Path.,* **20,** 429 (1944).
45. Armstrong, C.: Herpes simplex virus recovered from the spinal fluid of a suspected case of lymphocytic choriomeningitis. *Pub. Health Rep.,* **58,** 16 (1943).
46. Sabin, A. B., and Wright, A. M.: Acute ascending myelitis following a monkey bite, with the isolation of a virus capable of reproducing the disease. *J. Exp. Med.,* **59,** 115 (1934).
47. Sabin, A. B.: Studies on the B virus. I: The immunological identity of a virus isolated from a human case of ascending myelitis. *Brit. J. Exp. Path.,* **15,** 248 (1934).
48. Shope, R. E.: Experiments on the epidemiology of pseudorabies. II. Prevalence of the disease among middle western swine. *J. Exp. Med.,* **62,** 101 (1935).
49. Burnet, F. M., Lush, D., and Jackson, A. V.: The relationship of herpes and B viruses: immunological and epidemiological considerations. *Austral. J. Exp. Biol.,* **17,** 41 (1939).
50. Shope, R. E.: Experiments on the epidemiology of pseudorabies. I. Mode of transmission of the disease in swine and their possible rôle in its spread to cattle. *J. Exp. Med.,* **62,** 85 (1935).
51. Høygaard, A.: Acute epidemic diseases among Eskimos in Angmagssalik. *Lancet,* **1,** 245 (1939).
52. Theiler, M.: Spontaneous encephalomyelitis of mice, a new virus disease. *J. Exp. Med.,* **65,** 705 (1937).
53. Theiler, M., and Gard, S.: Encephalomyelitis of mice: I. Characteristics and pathogenesis of the virus. *J. Exp. Med.,* **72,** 49 (1940).
54. Armstrong, C.: Successful transfer of the Lansing strain of poliomyelitis virus from the cotton rat to the white mouse. *Pub. Health Rep.,* **54,** 2302 (1939).

55. SCHLESINGER, R. W., MORGAN, I. M., and OLITSKY, P. K.: Transmission to rodents of Lansing type poliomyelitis virus originating in the Middle East. *Science,* **98,** 452 (1943).
56. MELNICK, J. L.: Detection of the virus of mouse encephalomyelitis in the intestines of normal kangaroo rats. *J. Immun.,* **47,** 231 (1943).
57. OLITSKY, P. K.: A transmissible agent (Theiler's virus) in the intestines of normal mice. *J. Exp. Med.,* **72,** 113 (1940).
58. OLITSKY, P. K.: Further studies of the agent in intestines of normal mice which induces encephalomyelitis. *Proc. Soc. Exp. Biol. & Med.,* **43,** 296 (1940).
59. FRANCIS, T., JR., KRILL, C. E., TOOMEY, J. A., and MACK, W. N.: Poliomyelitis following tonsillectomy in five members of a family: an epidemiologic study. *J.A.M.A.,* **119,** 1392 (1942).
60. INTERNATIONAL COMMITTEE for the study of infantile paralysis: Poliomyelitis (Baltimore, 1932).
61. BURNET, F. M.: The epidemiology of poliomyelitis, with special reference to the Victorian epidemic of 1937–38. *Med. J. Australia,* **1,** 325 (1940).
62. LEE, R. K. C.: Statistics on poliomyelitis in the territory of Hawaii. *Pub. Health Rep.,* **56,** 1556 (1941).
63. KRUMWIEDE, C., MCGRATH, M., and OLDENBUSCH, C.: The etiology of the disease of psittacosis. *Science,* **71,** 262 (1930).
64. BEDSON, S. P.: Immunological studies with the virus of psittacosis. *Brit. J. Exp. Path.,* **14,** 162 (1933).
65. MEYER, K. F.: Ecology of psittacosis and ornithosis. *Medicine,* **21,** 175 (1942).
66. MEYER, K. F., EDDIE, B., and STEVENS, I. M.: Recent studies on psittacosis. *Am. J. Pub. Health,* **25,** 571 (1935).
67. BURNET, F. M.: Enzootic psittacosis amongst wild Australian parrots. *J. Hyg.,* **35,** 412 (1935).
68. BURNET, F. M.: A note on the occurrence of fatal psittacosis in parrots living in the wild state. *Med. J. Australia,* **1,** 545 (1939).
69. RASMUSSEN, R. K.: Er primaer epidemisk alveolo-pneumoni og psittacosis samme sygdom. *Ugeskrift f. Laeger,* **100,** 989 (1938).
70. HAAGEN, E., and MAUER, G.: Ueber eine auf den Menschen übertragbare Viruskrankheit bei Sturmvögeln und ihre Beziehung zur Psittakose. *Zentr. Bakt., 1 Abt., Orig.,* **143,** 81 (1938).
71. BLEYER, J. C.: Ueber Auftreten von Variola unter Affen der genera Mycetes und Cebus bei Vordringen einer Pockenepidemie. *Munch. med. Wchnschr.,* **69,** 1009 (1922).
72. COUNCILMAN, W. T.: Smallpox. *Modern Medicine,* edited by Osler and McRae (Philadelphia, 1907), **2,** 272.
73. FINDLAY, G. M.: Alastrim and its relation to variola. *Handbuch d. Virusforschung,* **2,** 864 (Wien, 1939).
74. GREENE, H. S. N.: Rabbit pox. III. Report of an epidemic with especial reference to epidemiological factors. *J. Exp. Med.,* **61,** 807 (1935).
75. WALDTEUFEL, and NOCARD: Quoted in F. Hutyra and J. Marek. *Special*

Pathology and Therapeutics of the Diseases of Domestic Animals. 3rd English Edition, **1,** 377 (1926).

76. Nagler, F. P. O.: Red cell agglutination by vaccinia virus. *Austral. J. Exp. Biol.,* **22,** 29 (1944).
77. North, E. A.: A study of the immunological reactions of the variola and vaccinia viruses grown in the developing egg. *Austral. J. Exp. Biol.,* **22,** 105 (1944).
78. Brinckerhoff, W. R., and Tyzzer, E. E.: Studies upon experimental variola and vaccinia in Quadrumana. *J. Med. Res.,* **14** (N.S. **9**), 209 (1905–06).
79. Creighton, C.: *History of Epidemics in Britain,* **2,** 434 (Cambridge, 1894).
80. Brown, C. J.: Smallpox in Port au Prince, Haiti. *U. S. Nav. Med. Bull.,* **15,** 695 (1921).
81. Ledingham, J. C. G.: The comparative study of clinically allied viruses: some unsolved problems of Edward Jenner. *Proc. Roy. Soc. Med. (London),* **29,** 73 (1935–36).
82. Nelson, J. B.: The stability of variola virus propagated in embryonated eggs. *J. Exp. Med.,* **78,** 231 (1943).
83. Horgan, E. S.: The experimental transformation of variola to vaccinia. *J. Hyg.,* **38,** 702 (1938).
84. Scott, H. H.: *A History of Tropical Medicine* (London, 1939).
85. Stokes, A., Bauer, J. H., and Hudson, N. P.: Transmission of yellow fever to Macacus rhesus. *J.A.M.A.,* **90,** 253 (1928).
86. Bauer, J. H.: Some characteristics of yellow fever virus. *Am. J. Trop. Med.,* **11,** 337 (1931).
87. Theiler, M.: Studies on the action of yellow fever virus in mice. *Ann. Trop. Med. & Parasitol.,* **24,** 249 (1930).
88. Theiler, M.: Neutralization tests with immune yellow fever sera and a strain of yellow fever virus adapted to mice. *Ann. Trop. Med. & Parasitol.,* **25,** 69 (1931).
89. Sawyer, W. A., and Lloyd, W.: The use of mice in tests of immunity against yellow fever. *J. Exp. Med.,* **54,** 533 (1931).
90. Soper, F. L., Rickard, E. R., and Crawford, P. J.: The routine post-mortem removal of liver tissue from rapidly fatal fever cases for the discovery of silent yellow fever foci. *Am. J. Hyg.,* **19,** 549 (1934).
91. Sawyer, W. A., and Whitman, L.: The yellow fever immunity survey of North, East and South Africa. *Trans. Roy. Soc. Trop. Med. & Hyg.,* **29,** 397 (1935–36).
92. Soper, F. L., Penna, H., Cardoso, E., Serafim, J., Jr., Frobisher, M., Jr., and Pinheiro, J.: Yellow fever without *Aëdes aegypti.* Study of a rural epidemic in the Valle do Chanaan, Espirito Santo, Brazil, 1932. *Am. J. Hyg.,* **18,** 555 (1933).
93. Soper, F. L.: Situation de la fièvre jaune au Brésil. *Bull. Off. internat. d'hyg. pub.,* **30,** 1205 (1938).
94. Balfour, A.: The wild monkey as a reservoir for the virus of yellow fever. *Lancet,* **1,** 1176 (1914).

95. Walcott, A. M., Cruz, E., Paoliello, A., and Serafim, J., Jr.: An epidemic of urban yellow fever which originated from a case contracted in the jungle. *Am. J. Trop. Med.,* **17,** 677 (1937).

96. Dudley, S. F.: Yellow fever as seen by the medical officers of the Royal Navy in the nineteenth century. *Proc. Roy. Soc. Med.,* **26,** 443 (1932–33).

97. Whitman, L.: The multiplication of the virus of yellow fever in *Aëdes aegypti. J. Exp. Med.,* **66,** 133 (1937).

98. Kirk, R.: An epidemic of yellow fever in the Nuba mountains, Anglo-Egyptian Sudan. *Ann. Trop. Med. & Parasitol.,* **35,** 67 (1941).

99. Soper, F. L., and de Andrade, A.: Studies of the distribution of immunity to yellow fever in Brazil. *Am. J. Hyg.,* **18,** 588 (1933).

100. Martini, E.: Tiergeographische Gesichtspunkte zur Beurteilung der Geschichte und Epidemiologie des gelben Fiebers. Abstract in *Trop. Dis. Bull.,* **27,** 865 (1930).

101. Dinger, J. E., Schüffner, W. A. P., Snijders, E. P., and Swellengrbel, N. H.: Onderzock over gele koorts in Nederland. *Nederl. tijdschr. v. geneesk.,* **73,** 5982 (1929).

102. Mathis, M.: Biologie comparée en conditions expérimentales, de quatre souches du Moustique de la fièvre jaune. *Compt. rend. Soc. de biol.,* **117,** 878 (1934).

103. Dudley, S. F.: Can yellow fever spread into Asia? An essay on the ecology of mosquito-borne disease. *J. Trop. Med. & Hyg.,* **37,** 273 (1934).

104. Paul, J. H., and Freese, H. L.: An epidemiological and bacteriological study of the "common cold" in an isolated Arctic community (Spitsbergen). *Am. J. Hyg.,* **17,** 517 (1933).

105. Hyde, R. R., and Chapman, J.: The behavior of certain filterable agents from cases of colds and influenza isolated directly from man to chick membranes. *Am. J. Hyg.,* **26,** 116 (1937).

106. Stuart-Harris, C. H., Andrewes, C. H., Smith, W., Chalmers, D. K. M., Cowen, E. G. H., and Hughes, D. L.: A study of epidemic influenza: with special reference to the 1936–7 epidemic. *Med. Res. Council, Sp. Report Ser.,* No. 228 (London, 1938).

107. Burnet, F. M., Cade, J. F. J., and Lush, D.: The serological response to influenza virus infection during an epidemic, with particular reference to subclinical infection. *Med. J. Australia,* **1,** 397 (1940).

108. Burnet, F. M., and Clark, E.: Influenza. *Monographs from Walter & Eliza Hall Institute,* No. 4 (Melbourne, 1942).

109. Shope, R. E.: The swine lungworm as a reservoir and intermediate host for swine influenza virus. I. The presence of swine influenza virus in healthy and susceptible pigs. II. The transmission of swine influenza virus by the swine lungworm. *J. Exp. Med.,* **74,** 41, 49 (1941).

110. Burnet, F. M., and Bull, D. R.: Changes in influenza virus associated with adaptation to passage in chick embryos. *Austral. J. Exp. Biol.,* **21,** 55 (1943).

111. Burnet, F. M.: Influenza virus on the developing egg. I. Changes asso-

ciated with the development of an egg-passage strain of virus. *Brit. J. Exp. Path.*, **17,** 282 (1936).

112. Burnet, F. M., and Lush, D.: Influenza virus on the developing egg. 8. A comparison of two antigenically dissimilar strains of human influenza virus after full adaptation to the egg membrane. *Austral. J. Exp. Biol.*, **16,** 261 (1938).
113. Burnet, F. M., and Bull, D. R.: Re-examination of the influenza virus strain "Melbourne egg." *Austral. J. Exp. Biol.*, **22,** 173 (1944).
114. Burnet, F. M.: The affinity of Newcastle disease virus to the influenza virus group. *Austral. J. Exp. Biol.*, **20,** 81 (1942).
115. Burnet, F. M.: Human infection with the virus of Newcastle disease of fowls. *Med. J. Australia,* **2,** 313 (1943).
116. French, C.: Report on the pandemic of influenza, 1918–19. *Reports on Public Health & Medical Subjects,* No. 4 (London: Ministry of Health, 1920).
117. Andrewes, C. H., Laidlaw, P. P., and Smith, W.: Influenza: Observations on the recovery of virus from man and on the antibody content of human sera. *Brit. J. Exp. Path.*, **16,** 566 (1935).
118. Burnet, F. M., and Lush, D.: Influenza virus on the developing egg. VII. The antibodies of experimental and human sera. *Brit. J. Exp. Path.*, **19,** 17 (1938).
119. Commission on Influenza: A clinical evaluation of vaccination against influenza. *J.A.M.A.*, **124,** 982 (1944).
120. Burnet, F. M.: Immunization against epidemic influenza with living attenuated virus. *Med. J. Australia,* **1,** 385 (1943).

DATE OF ISSUE

This book must be returned within 3, 7, 14 days of its issue. A fine of ONE ANNA per day will be charged if the book is overdue.

Class No. 116.9 Book No. A. S66 V

B. C. LIBRARY
PILANI

Author Arnaub

Title

Borrower's Signature P. C. D

Class & Address B. PH

Card No. 2662

Date